

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS



THE UNIVERSITY OF ALBERTA

PURIFICATION OF POTATO VIRUSES M AND S, AND BIOLOGICAL
INTERACTION IN THEIR RESPECTIVE LOCAL LESION HOSTS

by



ALLEN SHU-WING LAU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT SCIENCE


EDMONTON, ALBERTA

SPRING, 1977

To My Beloved Mother

and

My Late Father (1911-1972)



Digitized by the Internet Archive
in 2023 with funding from
University of Alberta Library

https://archive.org/details/Lau1977_0

ABSTRACT

Interaction between potato virus M (PVM) and potato virus S (PVS) was investigated using purified preparations of intact particles and ribonucleic acids (RNA's) in their respective local lesion hosts. The purification of PVM and PVS by differential precipitation with polyethylene glycol and sodium chloride resulted in increases in the virus yield up to 10-fold (330-350 mg virus per kg of infected leaves) over those obtained by the other methods reported in literature. The values of Absorbance 260/280 were found to be 1.46 ± 0.04 and 1.48 ± 0.03 for purified PVM and PVS respectively. The values of sedimentation coefficient and buoyant density of PVM and PVS were 108 and 148S, and 1.35 and 1.34 gm/ml respectively.

Inoculations of *Phaseolus vulgaris* L. cv. 'Red Kidney' or *Chenopodium quinoa* Willd. with a mixture of purified PVM and PVS respectively gave rise to marked increases in the local lesion number incited by a test virus, i.e. PVM or PVS. Neither PVM nor PVS mixed as a counterpart of a given test virus was recovered from the inoculated leaves of the non-host plant after mixed application. Approximately 10 to 30% increases in local lesion number incited by the test viruses were also obtained in the presence of the coat-proteins of the counterpart in the inoculum. Interaction of the viruses was not found when simultaneous separate inoculations were made onto the upper and lower epidermal layers of the test plants.

In the presence of the viral RNA of the counterpart, the

numbers of local lesions due to intact virus decreased 30 to 60%. Similarly, experiments with inoculum of mixed viral RNA's yielded 10 to 30% inhibition in the infectivity of the test viral RNA. This result suggested that there was no genetic interaction between these two viruses even at RNA level. The activity of the endogenous ribonuclease in the purified virus preparations was reduced by diluting the intact viral counterparts before mixing with the RNA preparations or by adding yeast-RNA to the viral RNA preparations when intact viral counterparts were mixed.

The results suggested that the stimulation of infection by a mixture of PVM and PVS was achieved possibly through the physical interaction of the particles between the intact viruses and even to a limited extent through an interaction with coat-protein of the counterpart. Thus, it was concluded that this type of interaction found between PVM and PVS in their respective local lesion host was a unique phenomenon different from any known interactions between different plant viruses.

ACKNOWLEDGEMENTS

I wish to thank Dr. C. Hiruki for his generous support and guidance throughout this study. Sincere thanks are also extended to Mr. T. Tribe for his valuable advices in photography, and Mr. H. Welling for his excellent care of the plants in the greenhouses.

I am indebted to Mr. M. Aarbo (Department of Biochemistry) for his kind help in performing the analytical centrifugations. Miss R. Chan's painstaking assistance in the preparation of illustrations is also gratefully acknowledged.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. LITERATURE REVIEW	11
Potato virus M (PVM) and potato virus S (PVS)	11
Distribution and economic importance	14
Purification	17
Interaction between different plant viruses	20
III. PURIFICATION AND PHYSICOCHEMICAL PROPERTIES	24
Introduction	24
Materials and Methods	25
Virus	25
Local lesion hosts	26
Purification	27
Electron microscopy	29
Analytical centrifugation	30
Buoyant density	30
Standard curves	31
Specific infectivity	31
Results	33
Discussion	37
IV. BIOASSAY	43
Introduction	43
Materials and Methods	45
Dark treatment	46
Post-inoculation temperature	46
Post storage temperature	46
Infectivity of PVM and PVS extracted from infected potato leaves after storage at different temperatures	46

Infectivity of PVM extracted from frozen infected potato leaves at various time intervals after incubation at 4° or at 24°C	47
Assay of PVM infectivity at different growth stages of infected potato plants	47
Reciprocal inoculation	47
Infectivity index	48
Results and Discussion	48
V. INTERACTION BETWEEN PVM AND PVS IN LOCAL LESION HOST	59
Introduction	59
Materials and Methods	59
Interaction between intact PVM and PVS	59
Simultaneous inoculation	60
Pre- and Post-inoculations	60
Simultaneous separate inoculations of upper and lower epidermal layers	60
Interaction between intact viruses, viral ribo- nucleic acids (RNA's), yeast-RNA and coat-proteins	61
Isolation of viral RNA's and coat-proteins	61
Interaction between intact virus and viral RNA of the counterpart	61
Simultaneous inoculation with PVM- and PVS-RNA's	61
Simultaneous inoculation with intact virus and viral coat-protein of the counterpart	61
Detection of endogenous ribonuclease (RNase) activity	62
Effect of endogenous RNase on PVS-RNA infectivity in the presence of yeast-RNA	62
Stimulatory index	62
Results	64
Discussion	88
VI. CONCLUDING REMARKS	92
PLATES AND LEGENDS	95
BIBLIOGRAPHY	102
APPENDIX	124

LIST OF TABLES

Table	Description	Page
1.	Comparison of some physical and biological properties of potato virus M (PVM), potato virus S (PVS) and other carlavirus members	36
2.	Effect of dark-treatment on the susceptibility of 'Red Kidney' bean (RKB) and <i>Chenopodium quinoa</i> to potato virus M and potato virus S, respectively	49
3.	Lesion counts: Infectivity of potato virus M and potato virus S, extracted from infected potato leaves, in 'Red Kidney' bean (RKB) and <i>Chenopodium quinoa</i> , respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls	54
4A.	The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. Expt. I	67
4B.	The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. Expt. II	68
5A.	The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to <i>Chenopodium quinoa</i> . Expt. I	69
5B.	The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to <i>Chenopodium quinoa</i> . Expt. II	70
6.	Stimulatory indexes obtained from the pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or <i>Chenopodium quinoa</i>	73
7.	Simultaneous separate inoculations with purified potato virus M (PVM) and potato virus S (PVS) of the upper and lower epidermal layers of 'Red Kidney' bean	75
8.	Simultaneous separate inoculations with purified potato virus S (PVS) and potato virus M (PVM) of the upper and lower epidermal layers of <i>Chenopodium quinoa</i>	76

9.	Interaction between intact potato virus M (PVM) and potato virus S (PVS)-ribonucleic acid (RNA) in 'Red Kidney' bean	79
10.	Interaction between intact potato virus S (PVS) and potato virus M (PVM)-ribonucleic acid (RNA) in <i>Chenopodium quinoa</i>	80
11.	Simultaneous application of potato virus M (PVM)- and potato virus S (PVS)-ribonucleic acids (RNA's) to 'Red Kidney' bean	82
12.	Simultaneous application of potato virus S (PVS)- and potato virus M (PVM)-ribonucleic acids (RNA's) to <i>Chenopodium quinoa</i>	83
13.	Simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) coat-protein to 'Red Kidney' bean	84
14.	Simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) coat-protein to <i>Chenopodium quinoa</i>	85
15.	The effect of endogenous ribonuclease associated with the purified potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in <i>Chenopodium quinoa</i> in the presence of yeast-RNA	87
16.	Lesion counts: The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean	125
17.	Lesion counts: Infectivity of potato virus M extracted from frozen infected potato leaves in 'Red Kidney' bean at various time intervals after their incubation at 4° or at 24°C	126
18.	Lesion counts: Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants	127
19A.	Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean. Expt. I	128
19B.	Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean. Expt. II	131
20A.	Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus S (PVS) in <i>Chenopodium quinoa</i> . Expt. I	135

- 20B. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus S (PVS) in *Chenopodium quinoa*. Expt. II 138
21. Lesion counts: The dilution curves of the infectivity of ribonucleic acids respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean (RKB) and *Chenopodium quinoa* 142
22. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM)-ribonucleic acid (RNA) in 'Red Kidney' bean 143
23. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa* 144

LIST OF FIGURES

Figure	Description	Page
1.	A flow diagram illustrating the purification procedure of potato virus M and potato virus S	28
2.	Standard curves of purified potato virus M (PVM) and potato virus S (PVS): ultraviolet absorbance at 260 nm was used as a measure of virus concentration	32
3.	Ultraviolet-absorption spectra of purified potato virus M (PVM) and potato virus S (PVS) preparations	34
4.	Buoyant density of potato virus M (PVM) determined by 20 to 40% cesium chloride (CsCl) density gradient centrifugation	38
5.	Buoyant density of potato virus S (PVS) determined by 20 to 40% cesium chloride (CsCl) density gradient centrifugation	39
6.	The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean	51
7.	Infectivity of potato virus M (PVM) and potato virus S (PVS), extracted from infected potato leaves, in 'Red Kidney' bean and <i>Chenopodium quinoa</i> , respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls	53
8.	Infectivity of potato virus M extracted from frozen infected potato leaves at various time intervals after their incubation at 4° or at 24°C	56
9.	Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants	58
10.	A standard curve showing the absorbance values of yeast-ribonucleic acid (RNA) of different concentrations determined at 258 nm	63
11.	Increases in the local lesion number of potato virus M obtained by applying a mixed inoculum of purified potato viruses M and S in different ratios to 'Red Kidney' bean	65
12.	Increases in the local lesion number of potato virus S obtained by applying a mixed inoculum of purified potato viruses S and M in different ratios to <i>Chenopodium quinoa</i>	66

13A.	Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or <i>Chenopodium quinoa</i> . Expt. I	71
13B.	Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or <i>Chenopodium quinoa</i> . Expt. II	72
14.	The UV spectra of ribonucleic acids (RNA's) respectively isolated from potato virus M (PVM) and potato virus S (PVS)	77
15.	The dilution curves of the infectivity of ribonucleic acids (RNA's) respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean and <i>Chenopodium quinoa</i>	78
16.	Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM)-ribonucleic acid (RNA) in 'Red Kidney' bean	86
17.	Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in <i>Chenopodium quinoa</i>	86

LIST OF PHOTOGRAPHIC PLATES

Plate	Description	Page
1.	Systemic and local lesion hosts of potato viruses M and S	96
2.	Electron micrographs and length-distribution of potato virus M (PVM) obtained by the polyethylene glycol-sodium chloride purification method	98
3.	Electron micrograph, length-distribution and cesium chloride (CsCl) isopycnic banding of potato virus S (PVS) obtained by the polyethylene glycol-sodium chloride purification method	100

LIST OF ABBREVIATIONS

Abbreviation	Vernacular name of the virus
AMV	alfalfa mosaic virus
CBV	chicory blotch virus
CLV	carnation latent virus
CMMV	cowpea mild mottle virus
CVB	chrysanthemum virus B
CV2	cactus virus 2
FMV	fressia mosaic virus
HLV	hop latent virus
LSV	lily symptomless virus
NLV	narcissus latent virus
PAMV	potato aucuba mosaic virus
PLV	passiflora latent virus
PMV	poplar mosaic virus
PSV	pea streak virus
PVA	potato virus A
PVM	potato virus M
PVS	potato virus S
PVX	potato virus X
RCVMV	red clover vein mosaic virus
STNV	satellite tobacco necrosis virus
TEV	tobacco etch virus
TMV	tobacco mosaic virus
TNV	tobacco necrosis virus
TYMV	turnip yellow mosaic virus
WBMV	white bryony mosaic virus

CHAPTER I

INTRODUCTION

Although the taxonomy of viruses is a highly controversial subject (Gibbs, 1969; Lwoff and Tournier, 1971), potato viruses M and S are now classified as members of the carlavirus group (Harrison et al., 1971), and this sigla grouping has been approved recently by the International Committee on the Taxonomy of Viruses (Fenner, 1976). The carlavirus group includes carnation latent virus (CLV) as its type member, and additional 11 members such as cactus virus 2 (CV2), chrysanthemum virus B (CVB), cowpea mild mottle virus (CMMV), lily symptomless virus (LSV), narcissus latent virus (NLV), passiflora latent virus (PLV), pea streak virus (PSV), potato virus M (PVM), potato virus S (PVS), red clover vein mosaic virus (RCVMV), and white bryony mosaic virus (WBMV). There are four more possible members suggested on the basis of partial characterization of the viruses. They include chicory blotch virus (CBV), fressia mosaic virus (FMV), hop latent virus (HLV), and poplar mosaic virus (PMV) (Brandes, 1964; Harrison et al., 1971). The members of the group are characterized by a particle length of 620-690 nm, straight to slightly flexuous rods, containing about 6% ribonucleic acid (RNA) (Gibbs, 1969; Harrison et al., 1971). Their longevity *in vitro* is only a few days at 20°C, the thermal inactivation point (TIP) is between 55°-70°C, and the

dilution end point (DEP) is 10^{-2} - 10^{-4} . They induce mild or no symptoms and have a narrow host range. Besides mechanical transmission, the viruses of this group may be transmitted by aphids. Their serological relationships range from nil to distant (Kassanis, 1955, 1956; Bagnall et al., 1956a,b; Rozendaal and van Slogteren, 1958; Bagnall et al., 1959; van Slogteren et al., 1962; Hakkaart et al., 1962; Brandes and Wetter, 1963-64; Wetter, 1967; Varma et al., 1970; Bos et al., 1972).

Soon after the turn of this century, Canada acquired an international reputation for high quality seed potato production through constant efforts in disease control and research in addition to climatic conditions suitable for such a purpose. Seed potato certification, started in Canada in 1913 by the National Plant Quarantine Act of 1912, aimed to restrict the spread of potato diseases across countries or continents (Rieman, 1956). By 1922, the certified acreage for seed potato production in Canada was 6,642 acres. Large scale serological and indicator plant assays began in 1952 to maintain seed potato stocks free from potato virus X (PVX) infection (Munro, 1954). Virus-tested potato stocks were established in British Columbia (Stace-Smith and Mellor, 1967, 1968a,b; Mellor and Stace-Smith, 1967, 1970, 1971; Wright, 1970; Wright and Mellor, 1976), and were introduced into elite seed farms in Eastern Canada in 1969 (MacKinnon and Bagnall, 1972). Approximately 85% of the certified seed potato acreage is in Prince Edward Island (P.E.I.) and New Brunswick (Seed Potatoes from Canada, 1975), and more than 75% of seed potatoes in Canada are grown annually in P.E.I. to meet the needs of both domestic and export markets (MacKinnon et al., 1972).

In Alberta today, the southern irrigated areas and the central region from Lacombe to Edmonton are the two major commercial potato farm lands (Holmes et al., 1976). The Alberta Seed Potato Improvement Program (ASPIP) was established in 1971 to supply the growers with disease-tested seed potatoes (Letal and Hiruki, 1976). Under this program seed potatoes, in particular Elites I and II, have been grown in isolated fields at Lacombe and Peers in Central Alberta. In 1976, while Alberta had 17,000 acres under potato cultivation, a 12% increase in yield was reported in the province (Fruit, Vegetable and Honey Crop and Market Report, 1976).

In 1976, Canada had 283,600 acres under potato production as compared to 260,000 acres in 1975 (Fruit, Vegetable and Honey Crop and Market Report, 1976). Of this, 65,000-71,000 acres were involved in the production of seed potatoes, and of which about 45,000 acres were for export purpose. Elite seed potatoes are maintained continuously by clonal selection, serological tests, indicator plant assays and other necessary tests to assure that they are free of tested viral, fungal or bacterial infection. At present, about 46 potato varieties are licensed for sale as seed potatoes (Seed Potatoes from Canada, 1975), and between 1964-1973, the annual export amounted to 125,000 tons.

Although Canadian seed potatoes have been exported to over 20 countries, the largest seed potato exporter in the world is the Netherlands. Van der Zaag (1972) reported that between 1965-1970 about 270,000 tons of certified seed potatoes were exported to more than 50 countries. Other European countries with significant export of seed potatoes are the United Kingdom, the Republic of Ireland, Denmark

and France.

Control of viruses in seed potatoes has had a long history. There are three principle methods, which have been used separately or in combination for better results: (1) varietal resistance, (2) thermotherapy, and (3) meristem tip culture.

Bagnall et al.(1956b) reported that certain cultivars including 'Saco', 'Alpha', 'Sebago', 'Voran' and 'Seedling X927-3' were found free from PVS infection by serological tests. Evidence of resistance to PVS in 'Saco' was reported also elsewhere (Alfieri and Stouffer, 1957; Bagnall et al., 1959; Alfieri, 1960; Bagnall and Young, 1960, 1968, 1972; Bagnall, 1965; Zadina, 1971). However, Larson and Oshima (1959) later found that when PVS was introduced to 'Saco' plants by top-graft inoculation the virus passed through the stem tissues into the roots where it could be detected. Baerecke (1967a) and Vulić and Hunnius (1967a) also reported transmission of PVS from infected scion to 'Saco' plants. Bagnall and Young (1960, 1969, 1972) hypothesized that resistance of 'Saco' potato was controlled by a single gene in the homozygous recessive condition, i.e. ssss being resistant to PVS while potato with a dominant gene 'S' is susceptible. Scholz (1964) obtained a PVS-resistant hybrid by crossing 'Ora' and 'Schwalbe' potatoes. Baerecke (1967b) and Makarov (1975) found that the resistance to PVS of a Bolivian clone, *Solanum tuberosum andigena* 'Huaca ñahui', Plant Introduction 258907, was due to the presence of a single dominant gene N_s . On screening 43 tuber-bearing *Solanum* species, Bagnall (1972) discovered that *S. microdontum* was resistant to both PVM and PVS.

Heat treatment has been a useful and an easily applicable

therapeutic method for controlling virus and mycoplasma diseases of plants. Effective treatment temperature and duration may vary from 35° to 54°C and from hours to weeks. Two kinds of potato materials have been used: the dormant tubers and young growing plants. The treatment of dormant tubers may rely strictly upon relatively simple heat-inactivation of the virus, however, hot-air-treatment of vigorously growing plants is more widely used.

The discovery of the frequent absence of tobacco mosaic virus (TMV) at the growing tip of infected plants (Limasset and Cornuet, 1949) consolidated Morel's (1948) pioneer work on the meristem tip culture technique to free plants from viruses. Hollings (1965) defined a meristem tip culture as an aseptic culture of apical meristem dome plus the first pair of leaf primordia, which is about 0.1-0.5mm long in different plants. Meristem tip culture alone often gives rise to virus-free clones, and is especially valuable when heat treatment fails (Bawden et al., 1950) or where plants cannot withstand heat. By growing the excised apical meristem (0.1-0.25mm) of sprouts from 'King Edward' and 'Arran Victory' infected with PVM and PVS, Kassanis (1957) obtained a limited number of virus-free plants. Similarly, Quak (1961), Yora and Tsuchizaki (1962), and Huth and Bode (1970) also produced few PVS-free potato plants. Morel and Muller (1964) eradicated PVS and PVX from 'Red Pontiac' and 'Eersteling' potatoes using the same technique with improved medium. Pett (1974) claimed, using meristem tip cultures, 84.6% of 'Axila' and 'Sperber' were made free from PVS, while 71.4% of 'Meise' were also free from PVM and PVS.

However, simple heat treatment or meristem tip culture alone

sometimes fails to produce virus-free potato plants. Virus-free nuclear stocks can be obtained more efficiently by combining thermotherapy and meristem tip culture techniques (Quak, 1972). Thomson (1958a) obtained some healthy potato plants by culturing PVS-infected shoot apices at 30°-38°C in darkness for 7-110 days, then excising and subculturing the shoot apices (1-2 cm). Similarly, Morel (1964) cultured meristem tips of sprouts from PVS-infected tubers germinated in darkness at 37°-38°C to obtain virus-free plants. Stace-Smith and Mellor (1968a,b) also succeeded in eliminating both PVS and PVX from infected 'White Rose' by growing the infected plants at 33°-37°C (with soil temperature at 30°- 32°C) for up to 7 months and culturing the excised axillary buds (0.3-1 mm, including 2-3 leaf primordia) on Murashige and Skoog's (1962) liquid medium. These techniques were soon applied to other infected stocks so that over 40 cultivars of potato licensed for sale in Canada were freed from PVS and PVX (Mellor and Stace-Smith, 1970; Wright, 1970; Wright and Mellor, 1976). Wright (1970) reported that PVS- and PVX-free 'Netted Gem' (Russet Burbank) and 'White Rose' seed potatoes increased their yield by 11-38% in British Columbia, Oregon and California. In fact, Shepard and Claflin (1975) stated that the only serious attempt being made in North America to produce PVS- and PVX-free certified seed potatoes at the grower level is in British Columbia, Canada.

Huth and Bode (1970) eliminated PVS from infected German potato varieties by heat treatment of tubers and meristem tip cultures, followed by grafting of plantlets onto tomato plants. In Finland, Tapio (1972) similarly reported an 80% recovery of potato plants free from PVS and PVX from apical meristems. Macdonald (1973)

in Scotland also recovered 58% of potato plants free from PVS and PVX when tested serologically.

Although much effort has been made in establishing virus-tested nuclear stocks, reinfection can occur. In nature, PVS can be transmitted by the aphid, *Myzus persicae* Sulz. (Bode and Weidemann, 1971; MacKinnon, 1974). After the introduction of virus-tested seed potatoes, 3-57% reinfection by PVS and PVX occurred in P.E.I. during 1969-1972, depending on the closeness of virus source and varietal susceptibility (MacKinnon et al., 1972; MacKinnon, 1974).

Nevertheless, there were two- to threefold increases in yield globally during the period 1935-1965 through improved health of seed potato stocks (Hollings, 1965). Shepard and Claflin (1975) reported three United States and one Canadian growing regions had recorded yield increases of 14-37% by using virus-tested seed potatoes. Between 1962-1973, 23% and 30% yield increases were reported from potato growing areas in the temperate and tropical zones in the world respectively (van der Zaag, 1976). Although the average yield of potatoes has been 13.3 tons/hectare, 95 tons/hectare has been achieved in experimental fields in the Netherlands. The survey from 1962 to 1973 indicated that the total acreage for world potato production will decrease continuously because of constant increase in yield due to the availability of disease-tested seed potatoes.

Bagnall et al. (1956a,b) determined serologically that PVM and PVS had a relatively small fraction of the antigenic determinants in common. On the basis of comparative serological studies on PVM, PVS and CLV, Kassanis (1956) and Bagnall et al. (1956b, 1959)

suggested that these three viruses might have had a common ancestry. Van Slogteren et al. (1962) and Hakkaart et al. (1962) proposed that the mutual degrees of serological relationships between PVM, PVS, CLV and CVB could be arranged as PVM-CLV-CVB-PVS in order of relatedness.

PVS was found with PVM in most commercial stocks of 'King Edward' potatoes (Bagnall et al., 1956b, 1959; Kassanis, 1956, 1960, 1961a; Rozendaal and van Slogteren, 1958; Howard and Wainwright, 1960; Bawden and Kassanis, 1964; Beemster and Rozendaal, 1972). Kassanis(1956) and Bawden and Kassanis (1964) reported that PVM and PVS could multiply, without interfering with each other, to much the same extent as if each of them were alone. Cross-protection, a phenomenon whereby serologically related viruses are mutually antagonistic and plants fully infected with one resist invasion by another, was not clearly observed between these two viruses (Kassanis, 1956; Bagnall et al., 1956b, 1959; Rozendaal and van Slogteren, 1958; Bawden and Kassanis, 1964; Beemster and Rozendaal, 1972), but different degrees of interference were reported between their strains by Rozendaal and van Slogteren (1958). These results suggest that the serological difference between PVM and PVS resulted in the apparent loss of their capability to interfere with each other. Bagnall et al. (1956b) found that a mixed inoculation of PVM and PVS resulted in the quick appearance of the chlorotic local lesions on *Datura Bernhardtii* Lund. and *D. metel* L., while PVS alone did not incite symptoms on either of the hosts, although it infected *D. metel* systemically.

On the other hand, Ambrosov and Sokolova (1973) reported that secondary infection with PVS significantly reduced infection by

PVM in the potato plants when determined serologically. Hunnius (1972), however, reported that the multiplication of PVS was inhibited by the secondary infection with PVM and later PVS was replaced completely. Howard and Wainwright (1960) and Bawden and Kassanis (1964) suggested that in mixed infections by PVM and PVS, the resulting symptoms depended largely on the virulence of the PVM strain. Hiruki (1970) found that there was a strong inhibitory effect by PVS on PVM infection in its local lesion host, *Phaseolus vulgaris* L. cv. 'Red Kidney', when a mixed inoculum in sap was used.

Therefore, the biological interaction between PVM and PVS remains as a controversial topic. One of the reasons for this was the lack of reliable bioassay systems for these viruses in the past. To determine the presence or absence of interference or interaction between the two viruses it is essential to analyze a mixed infection with highly purified viruses by the local lesion method or other quantitative techniques. In the past there were no such careful analyses made on these viruses. Although PVM and PVS have been known since 1923 and 1951 respectively, our understanding of their physicochemical properties is still fragmentary.

In the present study, investigations have been mainly directed towards the purification of potato viruses M and S in large quantities with improved yields. Using the highly purified virus preparations the physicochemical and biological properties of PVM and PVS have also been studied. The biological interaction of purified PVM and PVS was investigated at intact particle and RNA levels in their respective local lesion hosts. The effects of endogenous ribonuclease, coat-protein and yeast-RNA on the viral interaction have been included as

well in this study.

CHAPTER II

LITERATURE REVIEW

A. Potato Viruses M and S

Serious confusions in nomenclature occurred when earlier workers assigned different names for the same virus on the basis of symptoms, which might vary in severity under different conditions. A virus disease, believed to be caused by PVM, was first encountered by E. S. Schultz in 1923 and may serve as a typical example in this respect. Symptoms incited by PVM in 'Green Mountain' was first described under the name of leafrolling mosaic (Schultz and Folsom, 1923). Since then at least nine more synonyms have been assigned to this same virus (Rozendaal and van Slogteren, 1958; Wetter, 1972) as listed in the following: potato virus 7 (Johnson, 1927), potato paracrinkle virus (Salaman and Le Pelley, 1930), potato interveinal mosaic virus (McKay and Dykstra, 1932), potato virus E [unpublished thesis of F. C. Bawden (Smith, 1933)], Kartoffel-Rollmosaik-Virus (Kohler, 1935), Solanum virus 7 (Smith, 1937), Solanum virus 11 (Smith, 1937), *Marmor anglicae* Holmes (Holmes, 1939) and Kartoffel-K-Virus (Kohler, 1941). Bagnall et al. (1956b) designated the "interveinal mosaic virus (IVM factor)" of the 'Irish Cobbler' potato as "potato virus M", which at last won its universal acceptance.

PVS was accidentally discovered in 1948 at the Laboratory for

Flowerbulb Research at Lisse, the Netherlands, directed by E. van Slogteren during serological tests for potato virus A (PVA). Antiserum produced by using a supposedly pure PVA source from 'Light Industrie' not only reacted non-specifically with PVA alone, but also with an unknown viral antigen obtained from 'Light Industrie', 'Dark Industrie', and 'Bintje' regardless of the presence of PVA (van Slogteren, 1955a,b; Rozendaal and Brust, 1955). The field occurrence of this virus was first reported in 1951 by de Bruyn Ouboter (1952), and later was named as potato virus "S" in honor of its original discoverer, E. van Slogteren (Rozendaal, 1954; MacLeod, 1962).

The host range of potato viruses M and S includes several species in the families of Amaranthaceae, Chenopodiaceae, Leguminosae, and many species belonging largely to the Solanaceae (Bagnall et al., 1956b, 1959; MacLeod, 1962; Wetter, 1971, 1972). *Lycopersicon esculentum* Mill. (tomato) is immune to PVS, but systemically susceptible to PVM without producing visible symptoms. It may, therefore, be used as a filter plant to separate PVM from a mixed-inoculum of PVM and PVS (Rozendaal and Brust, 1955; Bagnall et al., 1956b; Kassanis, 1956; Vulić and Hunnius, 1967b; Horváth, 1972, 1973). Hiruki (1970) found that French bean 'Red Kidney' was also immune to PVS, suggesting that PVM could be separated from double-infected samples with subsequent subculture in tomato. Bagnall et al. (1956b, 1959) suggested that *Nicotiana debneyi* Domin. could be used as a test plant for the isolation of PVS since it is systemically susceptible to PVS while locally susceptible to PVM.

According to the cryptogram proposed by Gibbs et al. (1966) and Gibbs and Harrison (1968), PVM and PVS are described as "*/* : */* :

E/E : S/Ap" and "R/l (Hinostroza-Orihuea, 1973) : */* : E/E : S/Ap" [Type of nucleic acid (NA)/Strandedness : MW of NA/% NA : Outline of particle/Shape of nucleocapsid : Hosts/Vector] respectively, where E = elongated with parallel sides, ends not rounded, S = seed plant, Ap = aphid, R = ribonucleic acid, and l = single. Both viruses are straight to slightly flexuous filamentous particles of approximately 650 x 12 nm in size (Wetter and Brandes, 1956; Brandes et al., 1959; De Bokx, 1969; Tu and Hiruki, 1970; Hiruki and Shukla, 1973). PVM has a TIP of 65°-71°C (Wetter, 1972) while PVS's is 55°-60°C (Bagnall et al., 1956b; Wetter, 1971). Both viruses have a DEP of 10^{-2} - 10^{-4} (Bawden et al., 1950; Bagnall et al., 1956b; Wetter, 1971, 1972; Hiruki, 1973, 1975a), and their longevity *in vitro* is only 3 to 4 days at 20°C. Although Kassanis (1956, 1961a) failed to transmit PVM in 'King Edward' of the Rothamsted stock by aphids, Wetter and Völk (1960) succeeded in transmitting German isolates of PVM by *M. persicae* in other potato cultivars. It appears that different strains of PVM vary considerably in their aphid transmissibility. Bode and Weidemann (1971) reported that the rate of transmission by *M. persicae* ranged from 1 to 82% with five PVM strains. Similarly, despite earlier reports to the contrary (Rozendaal and Brust, 1955; Kassanis, 1956) various isolates of PVS were transmitted in the range of 3.4% to 42% by *M. persicae* (Bode and Weidemann, 1971; MacKinnon, 1974). However, PVM and PVS were not transmitted via true seeds of various infected *Lycopersicon* species (Horváth, 1973) or potato cultivars (Shepard and Claflin, 1975; Goth and Webb, 1975). These viruses are mainly maintained from generation to generation through the tubers of infected potato plants. Before disease-tested seed

potatoes were available, farmers in the Netherlands tended to retain smaller tubers from the previous crop for seed purpose, and this practice helped the spread of these viruses (Rozendaal and Brust, 1955).

In potatoes, PVM incites symptoms ranging from very slight (e.g. 'King Edward') to severe (e.g. 'Arran Victory'). 'King Edward' infected with PVM is usually symptomless and occasionally shows slight ruffling and paling of leaves, while in other cultivars, various diffused interveinal mottling, mosaic, crinkling and rolling symptoms in leaves, and dwarfing of the plant are observed (Bagnall et al., 1956b). The symptoms incited by PVS in potatoes are downward rolling, rugosity, slight veinbanding, chlorosis of leaves and stunting of shoots. Chmulev (1974) and Ambrosov and Schchutskaya (1975) reported infections by these two viruses resulting in marked decreases in pigmentation and photosynthetic activity in potato plants, but with increased respiration and oxidase activity.

B. Distribution and Economic Importance

Potato ranks fifth as a staple crop for human consumption following wheat, rice, maize and barley (van der Zaag, 1976). The total world potato crop production at present has amounted to 290 million tons and of these, 150, 100, 30, 8 and 2 million tons have been used for human consumption, stockfeed, seed, starch- and alcohol-productions respectively. In North America, 80% of the total potato production have been used for human consumption and 50% of which are in processed forms. In Europe, however, 50% of the potatoes produced have been used as stockfeed.

PVM and PVS are of economic importance because they are widely

distributed wherever potatoes are grown. In North America, Schultz (1951) reported that PVM reduced the yield of three potato varieties by 13-18% in Maine. PVM was also found in Oregon (McKay and Dykstra, 1932; Bagnall et al., 1956a,b, 1959; Kahn et al., 1967). Infection by PVS did not affect the number of 'Sabago' tubers but reduced the tuber weight (Lim et al., 1966). Other reports of PVS occurrence included Wisconsin (Rozendaal and Brust, 1955; Bagnall et al., 1956a,b, 1959; Larson and Oshima, 1959), California (Gold and Oswald, 1955), Oregon (Vaughan and van Slogteren, 1956), New York (Alfieri, 1960), Michigan (Lim, 1967), and others (Kahn et al., 1967).

In Canada, PVM was isolated from 'King Edward' and some other cultivars in Ontario and New Brunswick (MacLeod, 1939, 1962; Bagnall et al., 1956a,b, 1959), and more recently in Alberta (Hiruki 1970, 1973; Tu and Hiruki, 1970). Wright (1970) estimated that losses due to PVS in British Columbia ranged from 10 to 30%, especially when other viruses such as PVM or PVX were present. Occurrence of PVS was reported in New Brunswick (Bagnall and Bradley, 1955; Bagnall et al., 1956a,b, 1959), British Columbia (Stace-Smith and Mellor, 1968a,b), P.E.I. (MacKinnon, 1974), and Alberta (Hiruki, 1975a).

In Central and South American countries including Mexico, Guatemala, Haiti, Colombia, Ecuador, Peru, Bolivia and Chile, the overall incidence of PVM and PVS infections in various potato cultivars was about 2 and 14% respectively (Kahn and Monroe, 1970). McKee (1964) established the presence of PVS in Peruvian potatoes and Mendoza (1967) found that about 17% of them were infected by PVS. More recently, Hinostroza-Orihuela (1973) revealed an average of 28%

infection with PVS in different Peruvian cultivars. In Argentina, Pontis and Feldman (1963) reported the first record of PVM infection in the country.

Although PVM is not as widespread as PVS in Western Europe, its presence has been reported in England (Salaman and Le Pelley, 1930; Bawden et al., 1950; Kassanis, 1956, 1960, 1961a), West Germany (Köhler, 1941; Vulić and Hunnius, 1967b), France (Payen and Madec, 1957), the Netherlands (Rozendaal and van Slogteren, 1958), Italy (Lovisolo and Benetti, 1959), U.S.S.R. (Kolobaev, 1963), East Germany (Scholz, 1965a), Rumania (Ghena, 1970), and Poland (Chrzanowska, 1973, 1976). There are strong indications that PVM is now the most important virus of potatoes in Eastern Europe including U.S.S.R. (Hiruki, personal communication). Yield losses due to PVS were estimated to be 10-20%, depending on potato cultivar as well as virus strain (Rozendaal and Brust, 1955; van Slogteren, 1955c; Vaughan and van Slogteren, 1956; Kameraz and Shcherbakova, 1957; Beemster and Rozendaal, 1972). In the Netherlands, many potato cultivars have been much more severely infected with PVS than with PVX (Rozendaal and Brust, 1955; Rozendaal and van Slogteren, 1958). Widespread occurrence of PVS in various potato cultivars has also been reported in West Germany (Köhler, 1953, 1955; Wetter and Brandes, 1956), Scotland (MacArthur, 1956), France (Spire et al., 1967), Denmark (Kristensen, 1956), Finland (Aura, 1957; Tapio, 1972), Italy (Lovisolo and Benetti, 1959), England (Kassanis, 1961a), U.S.S.R. (Kameraz and Shcherbakova, 1957), Czechoslovakia (Jermoljev and Průša, 1958; Šíp and Havlová, 1969; Šíp, 1974), East Germany (Scholz, 1962, 1965a, b), Bulgaria (Bailova-Yankulova, 1966), Rumania (Ghena, 1967,

1970), and Hungary (Horváth, 1972).

Similarly, PVM and PVS were also found in Asia. Both viruses occurred in Japan (Oshima and Sato, 1965; Horio et al., 1969; Horio and Kumoi, 1975). Horio et al. (1969) reported that PVM infection in seed potatoes ranged from 11 to 100% in seven cultivars tested. PVM and PVS were present in a number of Korean potato seed stocks (La, 1974). Several PVM strains also were detected in Taiwan (Lee, 1971). The spread of PVS in India was studied by Ganguly (1963) and by Upreti and Nagaich (1969).

Despite the known occurrence of PVS (Sampson and Taylor, 1968), PVM has not yet been found in Australia (D.S. Teakle, personal communication). PVS has been identified serologically in three potato cultivars in New Zealand (Thomson, 1959). In South Africa, Herold (1967) found that 16% of various potato cultivars were infected with PVS.

C. Purification

Purification of the members of the carlavirus group has always been difficult in the past because of the low virus concentration in host tissues and losses due to aggregation of virus particles during isolation processes. Filamentous viruses are known for their frequent formation of insoluble aggregates, especially during concentration steps (Clark and Lister, 1971; Hödrejörv et al., 1970; Paliwal and Tremaine, 1976). These difficulties have considerably limited studies of physicochemical properties of the viruses belonging to the carlavirus group.

Polyethylene glycols (PEG) (trade name "Carbowax" is referred

to solid PEG with MW over 1000 dalton), uncharged linear polymers having a general formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, are chemically inert and their melting point, viscosity and aqueous solubilizing action all increase with increasing molecular weight (McClelland and Bateman, 1945). McClendon (1954) first reported that 20% (w/v) or higher Carbowax 4000 (MW 2400) together with 1M sodium chloride (NaCl) precipitated the "ultraviolet absorbing materials" from his chloroplast preparations. He signified that this discovery might provide a new tool in the study of nucleoproteins. Attempts were made initially to purify and concentrate viruses using aqueous two-phase systems of sodium-dextran-sulphate and PEG (Albertsson, 1960; Philipson et al., 1960). It was noted that the concentration of NaCl was significant in the system and it directly affected the final concentration of the virus in one phase or the other.

Hebert (1963) directly applied PEG 6000 and NaCl for precipitating plant viruses instead of the laborious conventional methods (reviewed by Steere, 1959). The two filamentous plant viruses he studied were precipitated by using 4% (w/v) or less PEG while the two spherical viruses were precipitated by 8% (w/v) PEG. The effect of PEG concentration on isometric virus precipitation was also studied later by Lastra and Munz (1969), who found a 90% recovery of the infectivity of squash mosaic virus by using 6% PEG 6000, while 4 and 2% PEG recovered only 70 and 40% of its total infectivity respectively. Venekamp and Mosch (1963; 1964a,b,c) and Leberman (1966) examined various concentrations of PEG 6000 and NaCl in cellulose column chromatography for virus purification. The virus could then be eluted with a buffer solution containing neither PEG nor NaCl. Using the same

technique Clark (1968) tried to precipitate alfalfa mosaic virus (AMV) with 8% (w/v) PEG 20000 and fractionated it on a kieselguhr column by washing with a 10% (w/v) nonlinear decreasing gradient solution of PEG 6000. Adomako et al. (1974) chromatographed cocoa necrosis virus that had been precipitated with 10% PEG 6000 on Celite and gel- (Sephadex G200, Sepharose 4B) filtration columns with decreasing concentrations of PEG (5, 3, 1, 0%) as eluent. This chromatographic purification was carried out with a belief that plant viruses could be precipitated and redissolved at different PEG and NaCl concentrations.

Investigation was made into the centrifugation of PEG-precipitated plant viruses through a reverse concentration PEG solubility gradient (10-0%), which was stabilized by a positive sucrose density gradient (5-30%) containing 0.15 M NaCl, for the determination of the minimal concentration of PEG and NaCl required for the precipitation of the virus or its components (Clark, 1970; Clark and Lister, 1971). In studying wound tumor virus, Reddy and Black (1973) reported both PEG and NaCl concentrations were very critical since they had a marked effect upon virus yield and specific infectivity.

Soon after Hebert's (1963) discovery, the PEG-NaCl precipitation procedure was widely adopted for the purification of not only other plant viruses but also animal viruses (Kanarek and Tribe, 1967; Wagner et al., 1970; McSharry and Benzinger, 1970; Freidmann and Haas, 1970) and Bacteriophages (Yamamoto et al., 1970). Recent reports involving PEG-NaCl purification of plant viruses are: pea seed-borne mosaic virus (Knesek et al., 1974), carrot thin-leaf virus (Howell and Mink, 1976), ryegrass mosaic virus (Paliwal and Tremaine, 1976), soybean dwarf virus

(Kojima and Tamada, 1976), tobacco mosaic virus (cowpea strain) (Whitfeld and Higgins, 1976).

D. Interaction Between Different Plant Viruses

Information on plant virus interactions is scattered through a tremendously large volume of literature and has been reviewed frequently (Price, 1940, 1964; Bennett, 1951, 1953; Ross, 1957, 1959, 1974; Kassanis, 1963; Best, 1965; Köhler, 1969; Matthews, 1970; Loebenstein, 1972a,b; Gibbs and Harrison, 1976; Dodds and Hamilton, 1976). In dealing with virus diseases occurring in nature it is not unusual to find double, triple or even quadruple virus infections in plants. While strains of a virus are mutually antagonistic, both synergism and antagonism commonly occur between different viruses. These interactions are often expressed in symptomatology and/or results in an abnormal increase in one of the interacting viruses.

Kassanis (1963) and Price (1964) interpreted interference as a phenomenon whereby a virus or viral component interferes with the multiplication of an unrelated virus or its disease-producing capacities, and that cross protection is a term designated to interference between strains of a virus only. In addition, these two phenomena differ in such a way that cross protection is specific, often almost complete, relatively long lasting, and frequently reciprocal, but interference is non-specific, incomplete, short lasting and one directional. However, Loebenstein (1972b), with a more inclusive view after considering recent progress made in this area of research, defined the interference of plant viruses as "a phenomenon whereby the infection process of one

virus interferes with the multiplication of another virus or strain; or whereby a non-multiplying substance interferes via a host-mediated process, requiring the transcription mechanism of the cell, with the multiplication of a virus".

In this review more emphasis will be given to the interaction between different viruses. Firstly, in synergistic interaction, a double infection by TMV and PVX, which resulted in severe streak symptom in tomato plants, is perhaps the first example that is well-known in plant virology (Dickson, 1925; Vanterpool, 1926). In other double infections, severe symptoms also resulted from a combination of TMV and tobacco etch virus (TEV) (McWhorter and Price, 1949), potato aucuba mosaic virus (PAMV) and AMV (Oswald et al., 1955), or TMV and cucumber mosaic virus (Garces-Orejuela and Pound, 1957; Marrow and Migliori, 1971). Development of a new symptom such as local lesions, which was not produced by neither of individual viruses, was found upon concurrent infection with a mixture of TMV and PVX (Sadasivan, 1940; Ross, 1959). In mixed infection, the concentration of interacting viruses is also affected. For example, the concentration of dodder latent mosaic virus (DLMV) greatly increased with no change in the concentration of the secondary viruses, when tomato plants infected with DLMV was superinfected with TMV or TEV (Bennett, 1949). Similarly, an increase of PVX concentration up to 10-fold was detected in the presence of PVY in potato and tobacco plants; the increase was still higher in simultaneous inoculation than in separate inoculations made in sequence (Ross, 1950; Rochow and Ross, 1954, 1955; Thomson, 1958b; Goodman and Ross, 1972). Concurrent inoculation with TMV and PVX or using TMV as a challenge virus, an increase up to 5-fold in

PVX was reported (Zachos, 1957; Thomson, 1961). TMV concentration in barley was much higher in the presence of barley stripe mosaic virus than in single infection with TMV (Hamilton and Dodds, 1970; Dodds and Hamilton, 1972).

Secondly, a number of reports pertaining to antagonistic effect of mixed infection exist, in which the viruses and procedures similar to those mentioned above were used. In antagonistic infection with mixed viruses, reduction in virus concentration was often noted. For example, infection with TEV established in potato or tobacco plants interfered with the secondary infection with PVY or henbane mosaic virus, and in concurrent inoculation, the systemic infection by the latter two viruses was suppressed or almost completely replaced by TEV even if they had first become established in the hosts (Bawden and Kassanis, 1945). In simultaneous infection with PVX and PVY, the latter decreased as the amount of PVX increased (Ross, 1950, 1957; Hutton, 1952). When TMV and PVX were inoculated simultaneously on tomato plants, a decrease of 20 to 65% in TMV concentration was found (Rochow, 1954; Rochow and Ross, 1954; Zachos, 1957). The speed of lesion development and/or the size of lesions incited by one virus in the established presence of another one decreased significantly (Price, 1935; McKinney, 1941; Bode, 1948; Ross, 1953; Nitzany and Cohen, 1960; Nitzany and Sela, 1962; Ford, 1967; Wenzel, 1971; Kassanis et al., 1974; Hiruki, 1975b). By contrast, simultaneous inoculation with TMV and PVX resulted in increases in the lesion-size of TMV, whereas if PVX was pre-inoculated, the resulting effect was negligible (Ross, 1961).

An unprecedented type of interaction was reported to occur

between satellite tobacco necrosis virus (STNV) and tobacco necrosis virus (TNV) (Kassanis, 1968). In this system, the multiplication of STNV was completely dependent on the presence of TNV, and in mixed inoculation in tobacco or bean plants the concentration of TNV decreased depending on the amount of STNV present in the inoculum (Kassanis and Nixon, 1961; Kassanis, 1962; Kassanis and White, 1972).

The studies of interaction between viruses have recently been carried out in protoplasts using both strains (Otsuki and Takebe, 1974) and unrelated viruses (Otsuki and Takebe, 1973, 1976; Takebe et al., 1975).

Lastly, another feature of interference is the interaction of an insect non-transmissible virus which becomes eligible in the presence of an insect transmissible virus. Clinch et al. (1936) were the first to find that PAMV became transmissible by aphids in the presence of PVA; and later PAMV was discovered also transmitted together with PVY (Kassanis, 1961b). Smith (1945, 1946) reported that tobacco mottle virus could only be transmitted by *M. persicae* together with the aphid dependent tobacco veindistorting virus present in the double infected tobacco plants.

CHAPTER III

PURIFICATION AND PHYSICOCHEMICAL PROPERTIES

INTRODUCTION

The purification of PVM and PVS was initially attempted by van Slogteren (1955) and Rozendaal and van Slogteren (1958). Their method involved extraction of sap in the presence of sodium bisulphite and potassium cyanide, clarification of the sap with chloroform, and isolation of the viruses by differential centrifugation. Wetter (1960) then purified both viruses, after clarification of sap with ether and carbon tetrachloride, by differential and rate-zonal density gradient centrifugation followed by dialysis. Albrechtová and Klír (1968) reported that PEG 4000 was suitable for the precipitation of PVS, and later extended their work to the purification of PVM using PEG of different molecular weights and concentrations (35% solution of PEG 4000, 30% solution of PEG 6000, and 20% solution of PEG 15000) (Albrechtová and Klír, 1970).

In PVM purification Hödrejčár et al. (1970) homogenized infected leaves of *S. demissum* in the presence of chloroform. The resulting clarified sap was subjected to differential centrifugation. Electrophoresis of the partially purified virus was then carried out in a sucrose density gradient column. Hiruki et al. (1974) used

n-butanol and 2% (w/v) PEG 20000 without NaCl to purify PVM.

In PVS purification, Shepard (1970) clarified the virus-containing leaf homogenate with chloroform, subjected it to differential centrifugation and precipitated the virus with 5% (w/v) PEG 6000; the final purification was achieved by two additional cycles of differential centrifugation. Kozar and Sivers (1975) added 5% Triton X-100 to the phosphate buffer during homogenization of infected tomato leaves to increase the yield of PVS, which resulted in an 8-fold increase in serological titre of the virus preparation obtained.

Despite of the reports on the purifications of PVM and PVS in the past only a limited amount of information is available on the quantitative estimation of yield, specific infectivity and the physicochemical properties of both viruses. The purposes of the present study are: firstly, to purify these two viruses quantitatively by applying an improved PEG-NaCl precipitation technique and thus to provide large amounts of purified PVM and PVS for the later interaction studies; secondly, examination of the purity of the virus preparations by ultraviolet (UV) absorption, electron microscopy, analytical and buoyant density centrifugations and thirdly, determination of the specific infectivity of purified PVM and PVS by using their respective local lesion hosts.

MATERIALS AND METHODS

A. Virus

The Alberta isolate (AP-1) of PVM was propagated in 'King

Edward' potato. The infected plants were slightly stunted but otherwise appeared 'normal' (Plate 1, A). The Canadian 'isolate A' of PVS, free from infections with other viruses including potato viruses M, X, and Y (Hiruki, 1975a), was multiplied in 'Netted Gem' potato (Plate 1,A). In PVS-infected plants veinclearing and leafrolling symptoms were evident in the early stages of their growth. Freedom of the samples used in this study from PVX infection was ascertained by using *Gomphrena globosa* L. (Wilkinson and Blodgett, 1948). The potato plants were grown in 20-cm plastic pots containing a soil-mixture of 3 parts loam, 1 part peatmoss and 1 part coarse-particle-size sand, and fed with commercial fertilizer (28-14-14) at intervals of 2-4 weeks, depending on growth conditions. The infected potato leaves, excluding mid-ribs, were harvested just prior to the blossom stage (1.5-2.0 months), put in polyethylene bags (200gm/bag) and stored in a deep freezer at -63.5°C until use.

B. Local Lesion Hosts

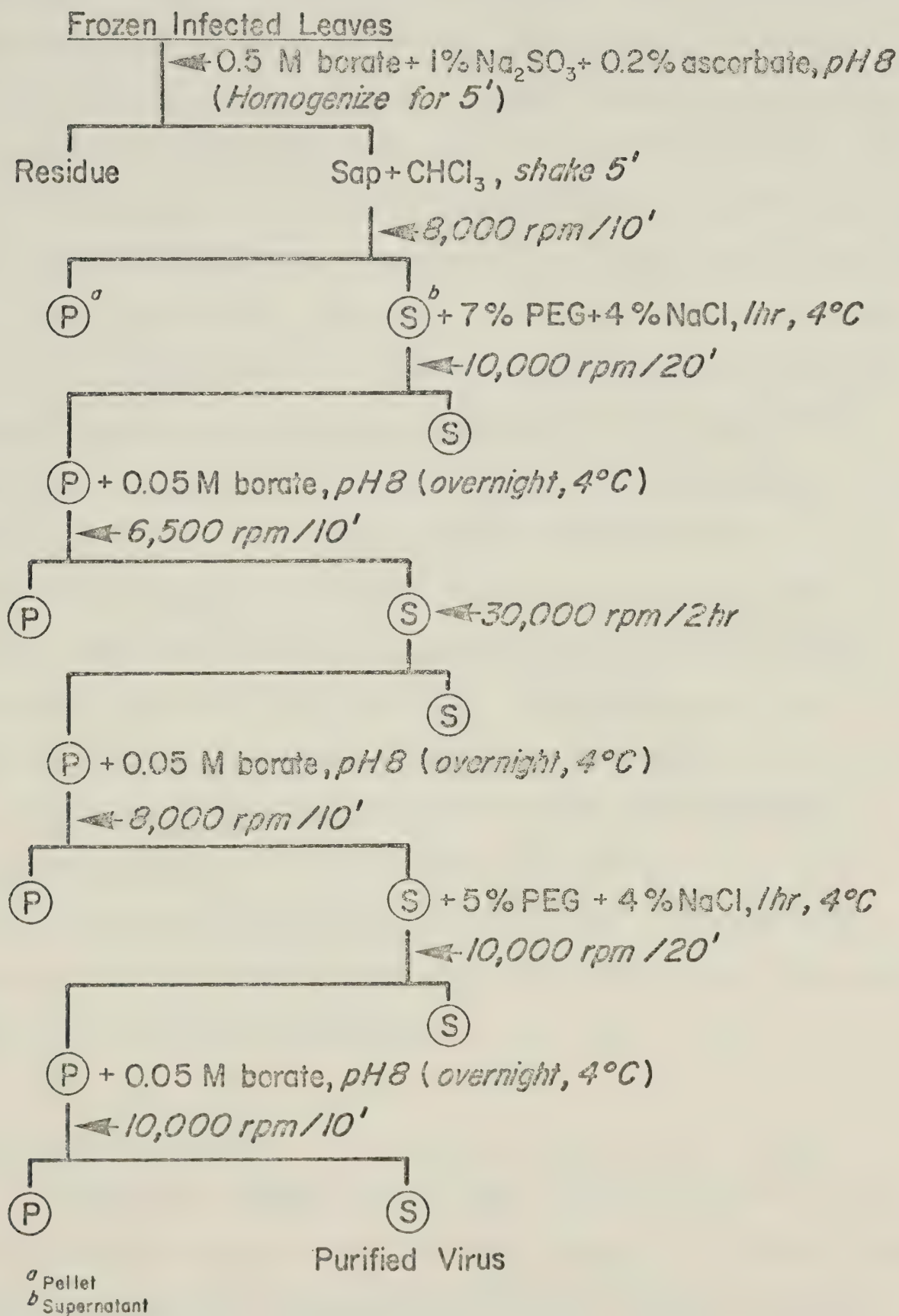
The selected seeds of 'Red Kidney' bean (RKB) (Town House brand, Empress Foods Ltd., Vancouver, B.C.) were sown in 10-cm clay pots containing steam-sterilized UC-mix II (C) (Matkin and Chandler, 1957). The plants were grown in a greenhouse at $17^{\circ}\pm 2^{\circ}\text{C}$ and cool-white fluorescent tubes at an intensity of 5,380 lx (500 ft-c) (Hiruki et al., 1974) were used to maintain a 16-hr photoperiod per day during the winter. Only those seedlings with the trifoliate leaf-bud just emerged were selected for PVM bioassay (Hiruki, 1970). The RKB plants of this particular growth-stage were obtainable from 9 to 12 days after seeding, depending on season of the year (Hiruki, 1973).

Chenopodium quinoa Willd. seeds, the C-selection (Hiruki, 1975a), were initially germinated in disposable plastic trays (12 cm X 19 cm X 6 cm deep) containing UC-mix. The seeds were covered with a thin layer (3-4 mm) of the soil-mixture. After 10 to 12 days the uniform seedlings were selected and transplanted individually into the clay pots containing the soil-mixture. The plants were grown in the greenhouse and fertilized at regular intervals. The greenhouses were properly shaded on the roof throughout the summer while a 16-hr photo-period per day was maintained with supplementary fluorescent and incandescent lights at an intensity of 16,000 lx in the winter. The *C. quinoa* plants were most susceptible to PVS during the period between 40-60 days after seeding (Hiruki, 1975a).

C. Purification

The following method was used for purifying both PVM and PVS. Frozen infected-leaves (200 gm) were homogenized in the presence of 400 ml buffer solution, which contained 0.5 M borate, 1% (w/v) sodium sulphite and 0.2% (w/v) L-ascorbate, adjusted to pH 8.0 with sodium hydroxide (NaOH), for 5 min using a Waring Blendor (Fig. 1). The green-colored juice was squeezed through two layers of cheese-cloth into a beaker kept in ice. The resulting sap sample amounting to 550-600 ml was transferred to a 2-litre volumetric flask and shaken vigorously with an equal volume of chloroform (Schneider, 1953) for 5 min. The emulsified mixture was transferred to six 250-ml polypropylene bottles and centrifuged at 8,000 rpm for 10 min (Sorvall model RC 2-B refrigerated centrifuge, GSA rotor). After the centrifugation three distinct layers were obtained. They were an amber-colored supernatant containing the

FIG. 1. A flow diagram illustrating the purification procedure of potato virus M and potato virus S.



virus, a whitish solid interphase coagulum (2.5-3.0 mm) of denatured protein and cell debris, and the chloroform solvent phase containing chloroplast-chlorophyll elements at the bottom. The supernatant was transferred into a 1-litre beaker kept in ice, to which a mixture of 7% (w/v) PEG 6000 (MW 6000-7500, J. T. Baker Chemical Co., N.J.) and 4% (w/v) NaCl was added with continuous stirring until dissolved. The solution was kept at 4°C for 1.0-1.5 hr (Wagner et al., 1970; Reddy and Black, 1973) to facilitate virus precipitation before being centrifuged at 10,000 rpm for 20 min. The virus in a pelleted form was suspended in 150 ml 0.05 M borate buffer adjusted to pH 8.0 with NaOH (hereafter referred to as borate buffer), overnight at 4°C. The suspension was centrifuged at 6,500 rpm for 10 min and the resulting supernatant was further centrifuged at 30,000 rpm for 2 hr (Beckman model L-4 preparative ultracentrifuge, Spinco rotor 30). The pellets thus obtained consisted of virus as a clear gelatinous top-layer and the chloroplast debris at the bottom-layer. The top-layer was dissolved in 40 ml of borate buffer and kept overnight at 4°C. The virus suspension was centrifuged at 8,000 rpm for 10 min and the supernatant was reprecipitated with 5% PEG and 4% NaCl. The pellet was dissolved in 7-8 ml of borate buffer and kept overnight at 4°C. The supernatant containing the purified virus was finally obtained by centrifuging the suspension at 10,000 rpm for 10 min.

D. Electron Microscopy

Droplets of diluted purified virus preparations were placed on 200-mesh Formvar-coated copper grids and a droplet of 2% neutral sodium phosphotungstate (PTA) was added to each sample (Brenner and Horne,

1959). A few seconds later the mixtures were blotted dry and the stained samples were examined immediately in a Philips 200 electron microscope at 60-80 kV. Leaf-dip preparations were also observed for comparison (Brandes, 1957).

E. Analytical Centrifugation

The sedimentation coefficient values of the purified PVM and PVS preparations were determined by the sedimentation-velocity method performed in a Beckman model E analytical centrifuge with built-in high intensity UV optical system and monochromater assembly. The preparations of PVM or PVS purified by the PEG-NaCl method were subjected to further purification by 5-30% (w/v) sucrose density gradient centrifugation (Brakke, 1951) at 25,000 rpm (Spinco SW25.1 rotor) for 2.5 hr, prior to the examination by analytical centrifugation. The resulting gradient samples were fractionated by an ISCO model D density gradient fractionator, dialysed by 5-7 changes of 0.1 M phosphate buffer containing mono- and di-basic potassium phosphates, pH 7.0, and then centrifuged at 30,000 rpm at 20°C in the analytical centrifuge. The sedimenting boundary was monitored with UV optics at 2 or 4 min intervals for 24 min and the microdensitometer tracings of the films were obtained. In some runs the progression of the sedimenting boundary was measured by using a photoelectric scanner and a multiplexer accessory. The sedimentation coefficient was calculated by the graphical method (Markham, 1960) and was corrected to the solvent viscosity of water at 20°C.

F. Buoyant Density

Solutions of 20, 30 and 40% (w/v) cesium chloride (CsCl) were made in 0.01 M Tris buffer containing 0.1 M NaCl and 1 mM ethylenediamine tetraacetate, pH 7.5. About 1 ml of the purified virus preparation was layered on the top of a discontinuous step gradient (Meselson et al., 1957) made by 0.5 ml 20%, 1.0 ml 30% and 1.5 ml 40% CsCl in a 5-ml Lusteroid tube, then the gradient tubes were centrifuged at 35,000 rpm for 20 hr at 4°C (Beckman model L-2 ultracentrifuge, Spinco SW 50.1 rotor). Fractions of 0.2 ml each were obtained from the gradient by piercing the bottom of the centrifuge-tube and the absorbance of each fraction was determined at 260 nm. The refractive index of selected fractions was also obtained (Erma Refractometer, Tokyo) and converted to the density of CsCl in gm/ml (Anderson and Anderson, 1970). The virus fraction was dialysed against 8-10 changes of borate buffer and assayed for infectivity in its respective local lesion host.

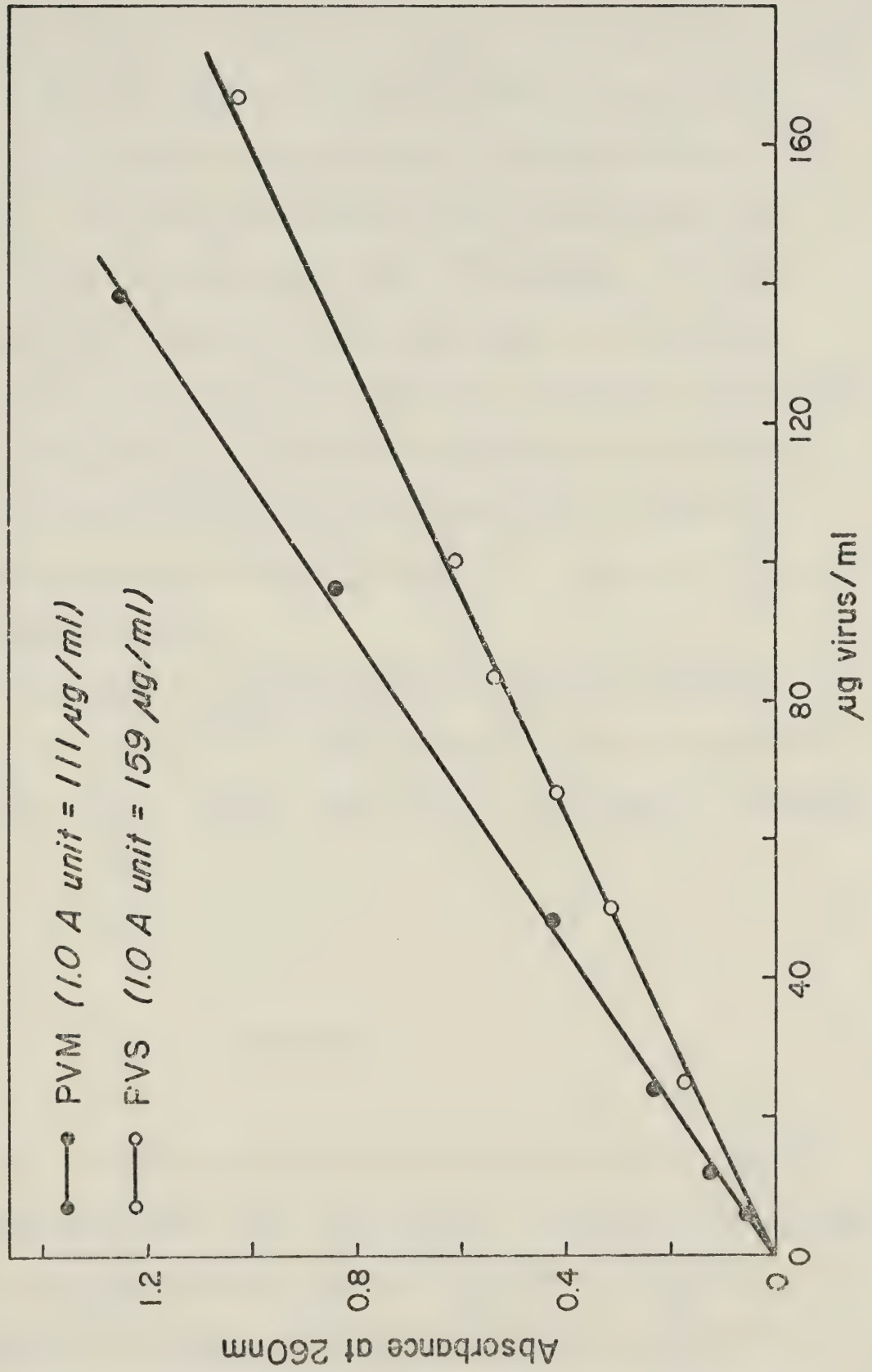
G. Standard Curves

PVM and PVS preparations purified by the PEG-NaCl method were further subjected to sucrose density gradient centrifugation (see Section F), dialysed against 10 changes of double distilled water, and were lyophilized in a freeze-dryer (Vir Tis Co., Inc., Gardiner, N.Y.) at -40°C for 4 days. A known weight of the lyophilized virus-preparation was dissolved in borate buffer to different concentrations for the determination of their absorbance (A) at 260 nm using a Hitachi Perkin-Elmer model 139 UV-VIS spectrophotometer (Fig. 2).

H. Specific Infectivity

Specific infectivity was determined by inoculating a respective

FIG. 2. Standard curves of purified potato virus M (PVM) and potato virus S (PVS): ultraviolet absorbance at 260nm was used as a measure of virus concentration.



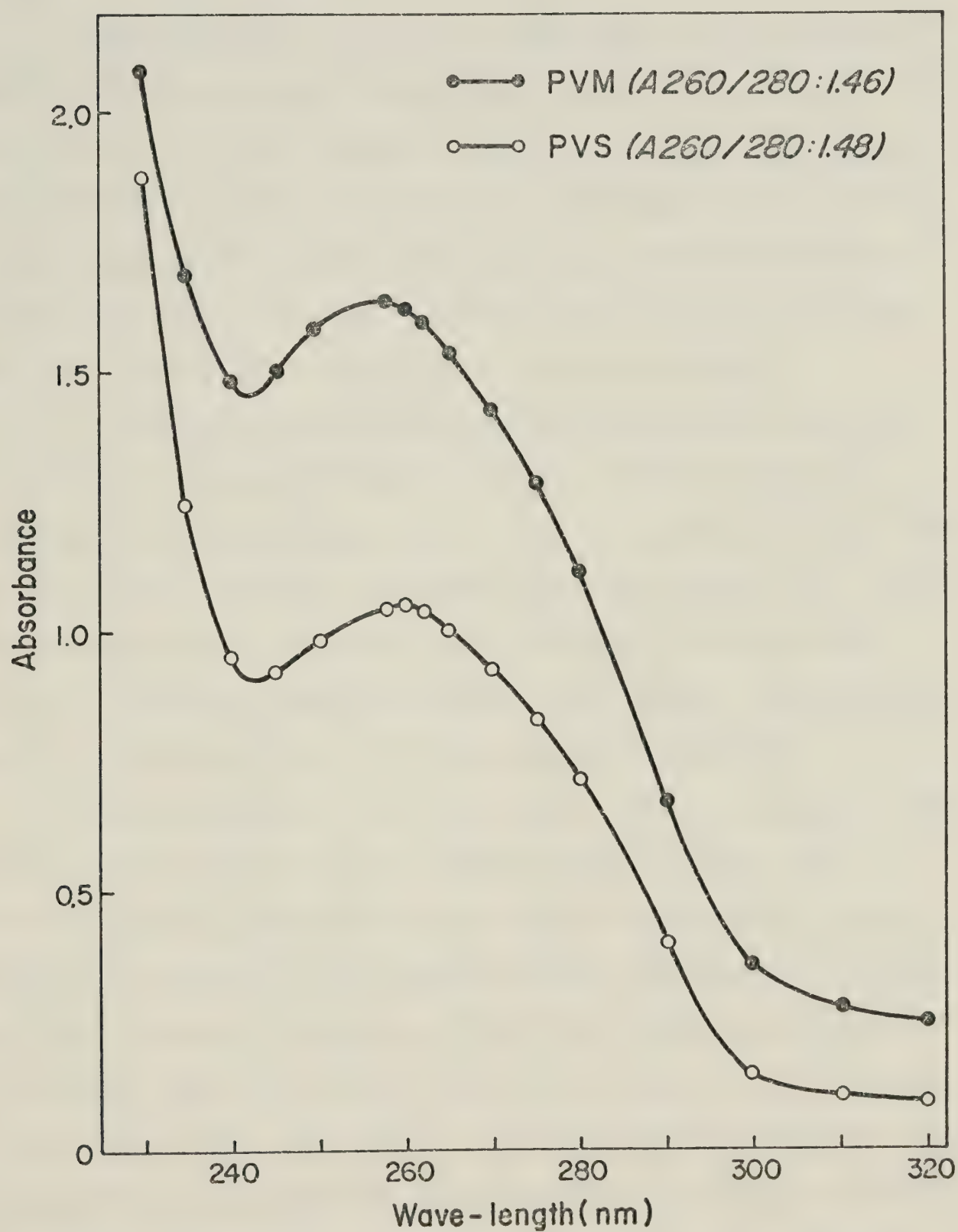
local lesion host with a known amount of purified PVM or PVS. The primary leaves of RKB were used for PVM assay, and before inoculation the buds of trifoliate leaves were removed. For PVS assay, 2-4 well-serrated leaves of each *C. quinoa* were selected for inoculation. The leaves were dusted uniformly with 600-mesh (20-22 μm) Carborundum (boron carbide, B_4C , Norton Co. Canada Ltd., Hamilton, Ontario) prior to inoculation. Inoculation was made by gently stroking the leaf surface with a Q-tip [Chesebrough-Pond's (Canada) Ltd., Toronto] dipped in inoculum. A piece of folded paper-towel of a convenient size was used to support the leaf at the under side during inoculation. Leaves were rinsed with distilled water immediately after inoculation. Two ml of virus suspension were applied separately to 62-64 half-leaves of RKB for PVM and 50-52 half-leaves of *C. quinoa* for PVS until the inoculum was exhausted.

The inoculated *C. quinoa* plants were incubated in growth chambers at $27^\circ\pm 2^\circ\text{C}$ with a 16-hr photoperiod of 10,760 lx (1,000 ft-c) light intensity (Hiruki, 1975a), while RKB were incubated as described previously.

RESULTS

Due to the combined strong anti-oxidizing effect of sodium sulphite (Bald and Samuel, 1934) and ascorbic acid (Best, 1939; Fulton, 1974) the extracted sap remained essentially green as expected. Purified preparations showed a UV-absorption spectrum, typical of purified virus preparations (Fig. 3). The values of $A_{260/280}$ were

FIG. 3. Ultraviolet-absorption spectra of purified potato virus M (PVM) and potato virus S (PVS) preparations.



1.46 \pm 0.04 and 1.48 \pm 0.03 for PVM and PVS respectively, which were within the range of the reported values for the carlavirus group (Table 1). The average virus yields were about 330 mg for PVM and 350 mg for PVS per kg of infected leaves.

Brown necrotic lesions (0.4-0.7 mm) (Plate 1, B) incited by PVM appeared in 3-4 days in RKB and were counted 8-9 days after inoculation. As to PVS, chlorotic lesions first appeared in 5 days, then increased in size (1.0-1.5 mm) with a necrotic centre in 7 days (Plate 1,C), and the maximum lesion number was obtained in 8-9 days after inoculation. The specific infectivities for purified PVM and PVS were 3 and 55 local lesions per μ g virus respectively.

In electron microscopy the purified virus preparations were relatively free from host materials (Plate 2,A and B; Plate 3,A). However, in the length distribution of the virus preparations purified by the present method PVM showed three major peaks (Plate 2,C), while PVS formed a normal distribution pattern (Plate 3,B) with a modal length of 645-655nm (Wetter and Brandes, 1956; DeBokx, 1969; Hiruki and Shukla, 1973) when 300 particles were measured respectively.

The sedimentation coefficient ($s_{20,w}$) values, reported for the members of the carlavirus group, ranged from 136 to 172S (Table 1). In this investigation, the purified PVS preparation exhibited a single boundary in analytical centrifugation and the sedimentation coefficient was 148S. However, in the case of PVM, three sedimenting boundaries were noted, giving sedimentation coefficient values of 108, 99 and 90S respectively. This result seems to correlate with the histogram showing the particle length distribution of PVM (Plate 2,C), and may represent an artifact resulting from degradation of PVM during the prolonged

TABLE 1. Comparison of some physical and biological properties of potato virus M (PVM), potato virus S (PVS) and other carlaviruses members.

	PVM	PVS	Carlaviruses ^a
Sedimentation Coefficient ($s_{20,w}$) (Svedbergs)	108(?)	148	136-172
Buoyant Density in CsCl (gm/ml)	1.35	1.34	1.33 ^b
A 260/280	1.46	1.48	1.09-1.55
Yield (mg virus/kg infected-leaves)	336	356	2-40 ^c
Specific Infectivity (Local lesion no./ μg virus) of purified virus	3	55	—

^a Included are : carnation latent, chrysanthemum B, cowpea mild mottle, lily symptomless, narcissus latent virus, pea streak, red clover vein mosaic and poplar mosaic viruses (Source: C.M.I./A.A.B. Descriptions of Plant Viruses, 1970-1976).
^b Narcissus latent virus (Brunt and Barton, 1976).
^c Refer to the text for specific virus involved.

purification procedure used in this study. In equilibrium density gradient centrifugation, the virus preparations purified by the present method showed only a single light scattering band in the CsCl gradient (Plate 3,C) and a single UV-absorption peak (Figs. 4 and 5). The buoyant densities of PVM and PVS were found to be 1.35 and 1.34 gm/ml respectively (Figs. 4 and 5). The virus fraction recovered from the CsCl density gradient after exhaustive dialysis had extremely low infectivity for PVS (2.4 lesions/half-leaf in 24 half-leaves) and none for PVM when assayed on their respective local lesion hosts.

DISCUSSION

A precipitation method using PEG and NaCl has been applied as a simple but efficient means of purifying bacteriophages, animal and plant viruses (Leberman, 1966; Gooding and Hebert, 1967; Kanarek and Tribe, 1967; van Kammen, 1967; Albrechtová and Klír, 1968; Yamamoto et al., 1970; Hsu and Black, 1973). Furthermore, the ease in the application of the procedures, another advantage of using this method, made quantitative purification of unstable viruses possible.

In separate experiments carried out along with this investigation, several other methods for PVM and PVS purification from the literature (see Introduction section) were tried out, but resulted in the virus yields ranging from 2 to 30 mg per kg of infected leaves (Lau, unpublished data). Hödrejörv et al. (1970) were able to obtain a yield of 10-15 mg purified PVM (strain M₁₁) per kg of leaf material. Other reported data on the yield of carlaviruses were: 2-5 mg for CMMV

FIG. 4. Buoyant density of potato virus M (PVM) determined by 20 to 40% cesium chloride (CsCl) density gradient centrifugation.

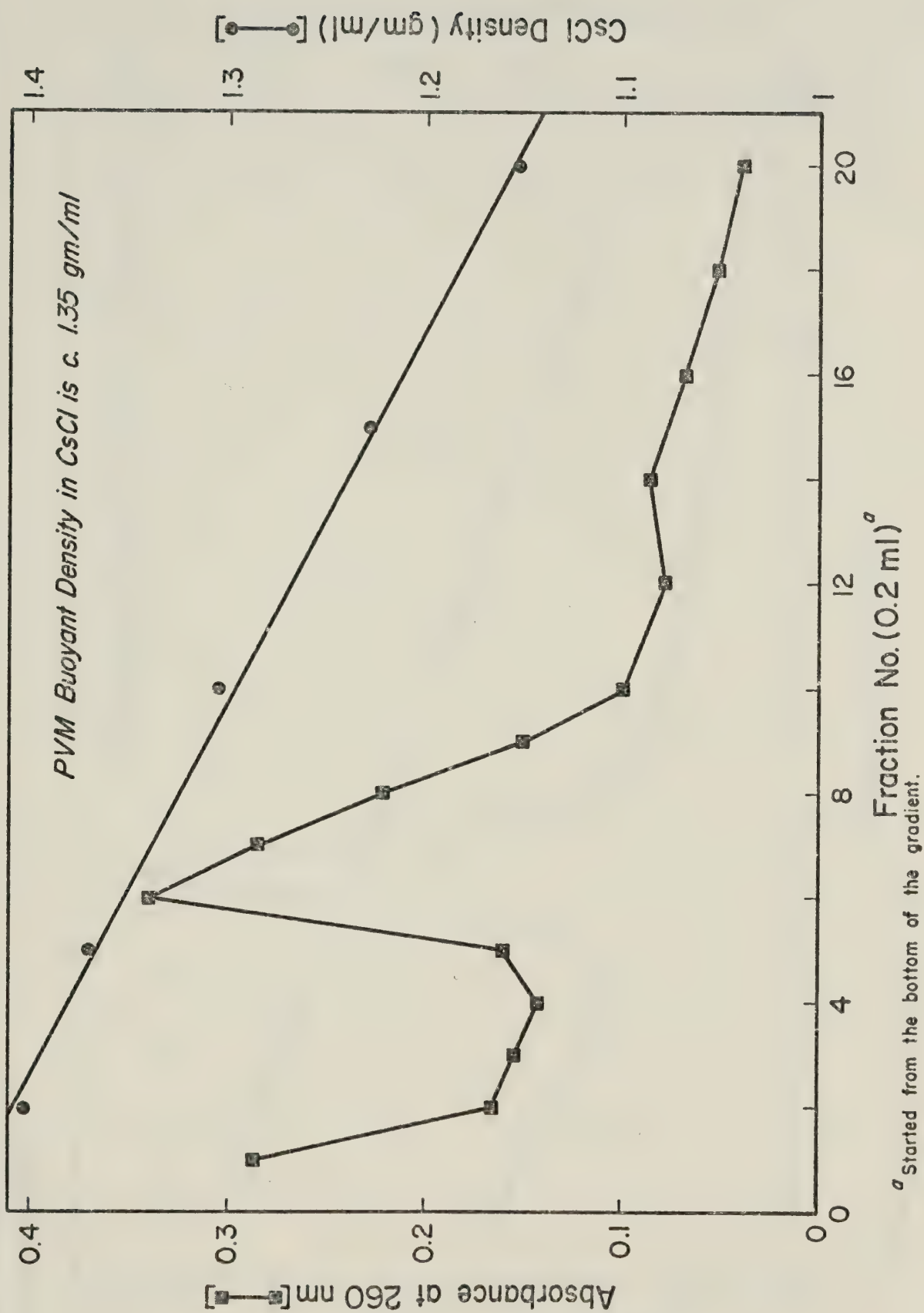
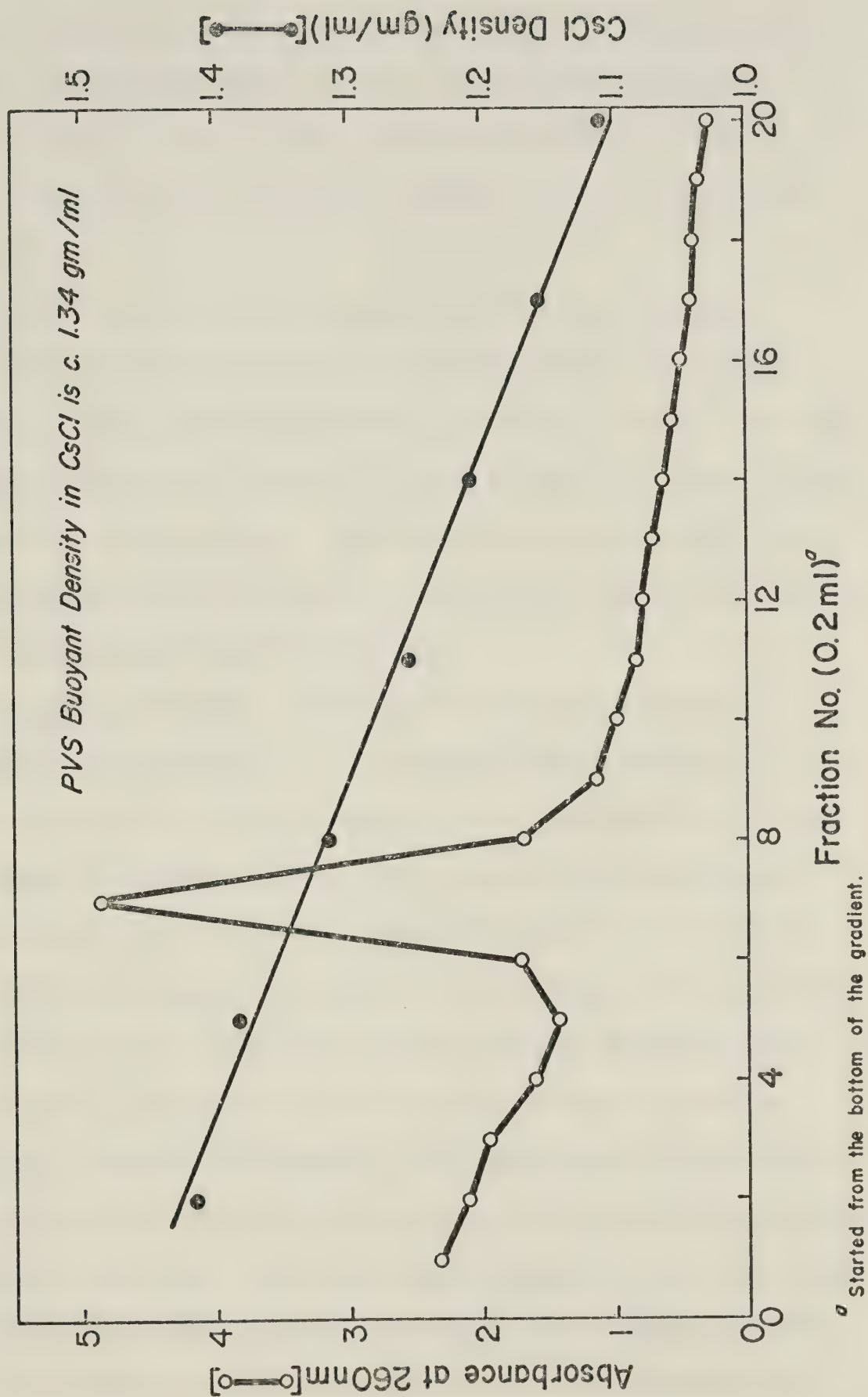


FIG. 5. Buoyant density of potato virus S (PVS) determined by 20 to 40% cesium chloride (CsCl) density gradient centrifugation.



(Brunt and Kenten, 1973), c.25 and 40 mg per kg of infected leaves for NLV and PMV respectively (Brunt and Barton, 1976). All these authors did not use the PEG-NaCl method in their purification procedures. In the present method, however, about a 10-fold increase in yield of purified viruses over the previously reported values for PVM and PVS was obtained.

In this study, the brief mixing of chloroform with sap containing PVS in the initial step of purification did not result in virus inactivation as reported previously with other viruses (Damirdagh and Shepherd, 1970; Howell and Mink, 1976). However, the same treatment resulted in much lower specific infectivity in the case of PVM, which may indicate some partial degradation of virus particles as revealed by electron microscopy and sedimentation data.

Paliwal and Tremaine (1976) reported that side-to-side aggregation of ryegrass mosaic virus particles was minimized by suspending the virus in buffer at pH 8.0. More aggregation was found at acidic than at alkaline pH with CMMV, another carlavirus member (Brunt and Kenten, 1973). In the present investigation, the use of alkaline buffer was also found suitable in preventing virus aggregation. When the purified virus preparations were suspended in borate buffer and stored at -63.5°C , homogeneous virus suspensions were consistently obtained after thawing. In contrast, virus aggregates occurred after thawing the purified virus preparations that had been suspended in double distilled water (pH 5.6). Kaper and Siberg (1969) reported that turnip yellow mosaic virus (TYMV), suspended in water and incubated at -75°C , dissociated into RNA and capsid when thawed, and they suggested the RNA's bound to the insoluble protein fragments forming aggregates.

The values of the buoyant density of both viruses were similar and were comparable to that of the NLV (Table 1), the only other carlavirus member whose buoyant density value has been reported recently (Brunt and Barton, 1976). Yet the sedimentation coefficient of PVM was much lower than that of PVS and those of certain other carlavirus members. Besides degradation, perhaps many empty particles, especially those having the "normal length" might be present in the purified PVM preparations. However, the data pertaining to the UV-absorption spectrum of the PVM preparations used in this investigation did not suggest the presence of such defective forms to the extent detectable.

In contrast to isometric viruses (Bancroft, 1962), CsCl isopycnic banding resulted in tremendous losses of virus infectivity and thus was unsuitable for the purification of PVM and PVS. The exposure of other filamentous viruses to certain heavy salt solutions resulted in marked aggregation, and in some cases even complete degradation of virus particles occurred (Damirdagh and Shepherd, 1970; Rao, 1977). In this investigation, the UV-absorption spectrum of PVM indicated that a light-scattering material was present beneath the meniscus (fraction no. 14), indicating the occurrence of degradation of virus particles (Fig. 4).

Unlike ammonium sulphate, the precipitation with PEG-NaCl is more selective and the virus could be precipitated regardless of its concentration. Furthermore, if ribosomes were present initially in the preparation, they were not precipitated unless the concentration of PEG 6000 was increased to 10%(w/v) or higher (Yamamoto et al., 1970). Thus, the purified virus preparations by the present method were

apparently free of ribosome contamination as indicated by electron microscopy (Plates 2,A and 3,A), analytical and buoyant density centrifugations. Shepard (1970) also reported a similar observation from the electron microscopy of the PEG-NaCl purified PVS and PVX preparations.

CHAPTER IV

BIOASSAY

INTRODUCTION

Bagnall et al. (1956b, 1959) reported that PVM incited irregular reddish spot or ring-like lesions on the inoculated primary leaves of *Vigna sinensis* Endl. (cowpea) 14 days after inoculation, and that *D. metel* produced chlorotic local lesions 8 days after inoculation prior to systemic chlorotic spotting. As a local lesion host RKB is useful for PVM bioassay (Hiruki, 1970, 1973; Dziewońska and Ostrowska, 1973; Hiruki et al., 1974). Ross (1968) and Kowalska and Waś (1976) reported *L. chilense* Dun. as a reliable indicator plant for PVM.

With regard to assay hosts for PVS, Yarwood and Gold (1955) reported *Cyamopsis tetragonaloba* (L.) Taub. (guar) as a local lesion host. However, later it was found that these lesions were identical with those of PVM (Bagnall et al., 1956b, 1959). Ross (1968) also reported *L. chilense* as a useful assay plant for PVS. PVS incited necrotic lesions on *Saracha umbellata* Don. (Bagnall et al., 1956b) and *C. album* L. responded with chlorotic spots in about 20 days after inoculation (Bagnall et al., 1956b, 1959; Scholz, 1965b; Vulić and Hunnius, 1967b). Loughnane (1958) found that *Beta macrocarpa* (annual beet) developed local lesions in about 20 days. *C. amaranticolor* Coste

and Reyn. was also found to be a local lesion host for PVS (Hollings, 1956), and Vulić and Hunnius (1967b) reported *C. quinoa*, *C. album*, *C. amaranticolor*, and *G. globosa* as the assay hosts for PVS in descending order of their sensitivity and efficiency. Other workers (Vulić and Hunnius, 1967b; de Bokx, 1970; Cupertino et al., 1970; Hiruki, 1975a; Kowalska and Waś, 1976) also agreed unanimously that *C. quinoa* was the useful local lesion host for PVS.

The optimal assay conditions for both local lesion host and virus inoculum have been studied extensively for PVM (Hiruki, 1970, 1973; Dziewońska and Ostrowska, 1973; Hiruki et al., 1974; Kowalska and Waś, 1976) and for PVS (Hiruki, 1975a; Kowalska and Waś, 1976) in respect to plant age, buffer concentration and pH, dilution curve, post-inoculation temperature and light intensity for maximal lesion development.

Environmental conditions, such as variations in illumination and temperature, influence the susceptibility of many host plants to virus infection. Bawden and Roberts (1947, 1948) discovered that pre-inoculation shading or darkening of test plants for 24 or 48 hr resulted from 2- to 10-fold increases in lesion number with 4 viruses tested. Subsequently, shading or darkening plants for 1-2 days before inoculation was employed routinely to increase the susceptibility of test plants with viruses that were difficult to transmit (Kassanis, 1949; Watson, 1952). At the same time, the significance of temperature effect on virus infectivity should not be overlooked. Post-inoculation temperature affected directly the local lesion production in assay hosts (Frost and Harrison, 1967; Hiruki, 1975a). Tobacco ringspot virus in sap was found inactivated after 1 day at 20°C, but retained its

infectivity for 85 days at -5°C (Priode, 1928), while tomato spotted wilt virus was inactivated in 3 hr at room temperature (Bald and Samuel, 1934). Bawden and Pirie (1950, 1957) found that freezing minced leaves or expressed sap did not inactivate TNV.

In the present study, the effects of dark treatment and post-inoculation temperature on the local lesion hosts, and post storage temperature effect on virus infectivity were investigated. In addition, assays of PVM infectivity at different growth stages of infected potato plants and the reciprocal inoculations of PVM and PVS on their respective local lesion hosts were carried out.

MATERIALS AND METHODS

Sap from infected potato leaves was extracted by using a mortar and pestle at a 1:10 (w/v) ratio in 0.057 M phosphate buffer containing mono- and di-basic potassium phosphates, pH 8.0 (hereafter referred to as phosphate buffer), as inoculum (Hiruki et al., 1974; Hiruki, 1975a) in all experiments described below. Inoculation procedure and incubation conditions were essentially the same as described before (Chapter III, Specific infectivity section). All experiments were done by using the half-leaf method with the balanced distribution of treatments wherever possible (Holmes, 1929; Samuel and Bald, 1933; Loring, 1937). Immediately after inoculation the leaf was tilted to one side for washing with distilled water from a wash-bottle to avoid contamination of the opposite half leaf.

A. Dark Treatment

Five vigorously growing 8-day-old RKB and 5 uniformly grown 40-day-old *C. quinoa* (2 leaves/plant) plants were placed in a ventilated darkchamber [internal measurement (cm): 113(length), 60(width) and 45 (depth)] and kept in a greenhouse for 48-hr dark treatment at $20^{\circ}\pm 2^{\circ}\text{C}$. On the second day, the same number of assay plants from the same batches were added for 24-hr dark treatment. On the third day, the treated plants along with the same numbers of untreated plants from the same batches were inoculated with sap samples containing PVM and PVS respectively.

B. Post-inoculation Temperature

RKB plants inoculated with PVM, 12 half-leaves per treatment, were placed separately in controlled growth chambers set at 17° , 22° , 27° , and 32°C , with a 16-hr photoperiod of 5,380 lx.

C. Post Storage Temperature

a. *Infectivity of PVM and PVS extracted from infected potato leaves after storage at different temperatures*

PVM-or PVS-infected potato leaves were harvested, cut, mixed, and divided into 3 batches of 0.2 gm each. The leaves were enclosed in polyethylene bags and one half of each virus sample was stored at 4°C for 6 days, while the remaining half was kept frozen at -63.5°C . After 3 days of storage at -63.5°C one of each virus sample was thawed at room temperature for 0.5 hr before being refrozen at -63.5°C . Infectivity of both viruses in the leaf samples from different temperature treatments was determined after 6-day-storage. Concurrently, fresh

sap extracted from the leaves of the same PVM- or PVS-infected potato plants were tested as controls.

b. Infectivity of PVM extracted from frozen infected potato leaves at various time intervals after incubation at 4° or at 24°C

PVM-infected leaves were harvested from a 'King Edward' plant, cut, mixed, and divided into 11 batches of 0.2 gm each. Each sample was enclosed in a polyethylene bag and stored immediately at -63.5°C for 6 days before removing 5 batches each for incubation at 4° or at 24°C. For a control at zero time, infectivity assay was carried out immediately after extraction. Samples incubated at 4° or at 24°C were tested for their infectivity at 0.5-, 2-, 8-, 24- and 48-hr intervals.

D. Assay of PVM Infectivity at Different Growth Stages of Infected Potato Plants

PVM-infected tubers of 'King Edward' were seeded as described before (Chapter II, Virus section). The first leaf samples, 0.2 gm each, were harvested from the first fully developed leaves 22 days after seeding. At the same time, the plant height and the height of leaves sampled were recorded. A total of 10 leaf samples was harvested at 3-day intervals, then individually enclosed in a polyethylene bag and stored immediately at -63.5°C until use.

E. Reciprocal Inoculation

A total of 24 half-leaves of RKB and *C. quinoa* were inoculated respectively with a sap sample of PVM from 'King Edward' leaves. After 9 days, the inoculated leaves of *C. quinoa* were harvested and ground for back inoculation on RKB. Leaf-dip preparations from the inoculated

C. quinoa leaves were prepared by sliding the freshly cut leaf edge across a droplet of 2% neutral PTA resting on a 200-mesh Formvar-coated copper grid, blotted dry, and examined immediately in a Philips EM200 at 60-80 kV. Similar duplicated tests also were carried out for PVS in the reversal sequence of inoculation of the local lesion hosts.

F. Infectivity Index

In the present study the following formula was used to obtain an infectivity index from local lesion counts.

$$\text{An infectivity index} = \frac{\text{Average number of local lesion obtained in test}}{\text{Average number of local lesion obtained in control}}$$

RESULTS AND DISCUSSION

A. Dark Treatment

The RKB plants that had been subjected to the dark treatment were pale in color and fragile upon touching. Two to 6-fold increases in susceptibility were demonstrated in local lesion number (Table 2). In both PVM and PVS infection the number of local lesions obtained was much higher in the 48-hr dark treatment. In an earlier experiment, where aluminum foil was used to cover the opposite half-leaf for dark treatment, no increase in susceptibility was observed (Hiruki et al., 1974). This might have been due in part to some possibilities that photosynthetic products as well as water are freely translocated throughout the plant so as to compensate the resulting effects of dark treatment in the covered portions of the leaves. Therefore, dark treatment of

TABLE 2. Effect of dark-treatment on the susceptibility of 'Red Kidney' bean (RKB) and *Chenopodium quinoa* to potato virus M and potato virus S, respectively.

Local lesion host ^a	Plant no.	Control ^b	Dark-treatment ^c	
			24 hr	48 hr
R K B	1	35	74	196
	2	28	35	153
	3	20	32	231
	4	32	51	94
	5	16	32	138
Lesion no./half-leaf		6.6	11.2	40.6
Standard error		0.9	2.0	5.9
Infectivity index		1.0	1.7	6.2
<i>C. quinoa</i>	1	172	545	1256
	2	193	836	1101
	3	145	796	955
	4	322	1051	1613
	5	186	659	1321
Lesion no./half-leaf		50.9	194.4	312.3
Standard error		7.7	21.5	27.7
Infectivity index		1.0	3.8	6.1

^a Four half-leaves per plant were inoculated.

^b Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

^c Dark-chamber temperature: $20 \pm 2^\circ\text{C}$.

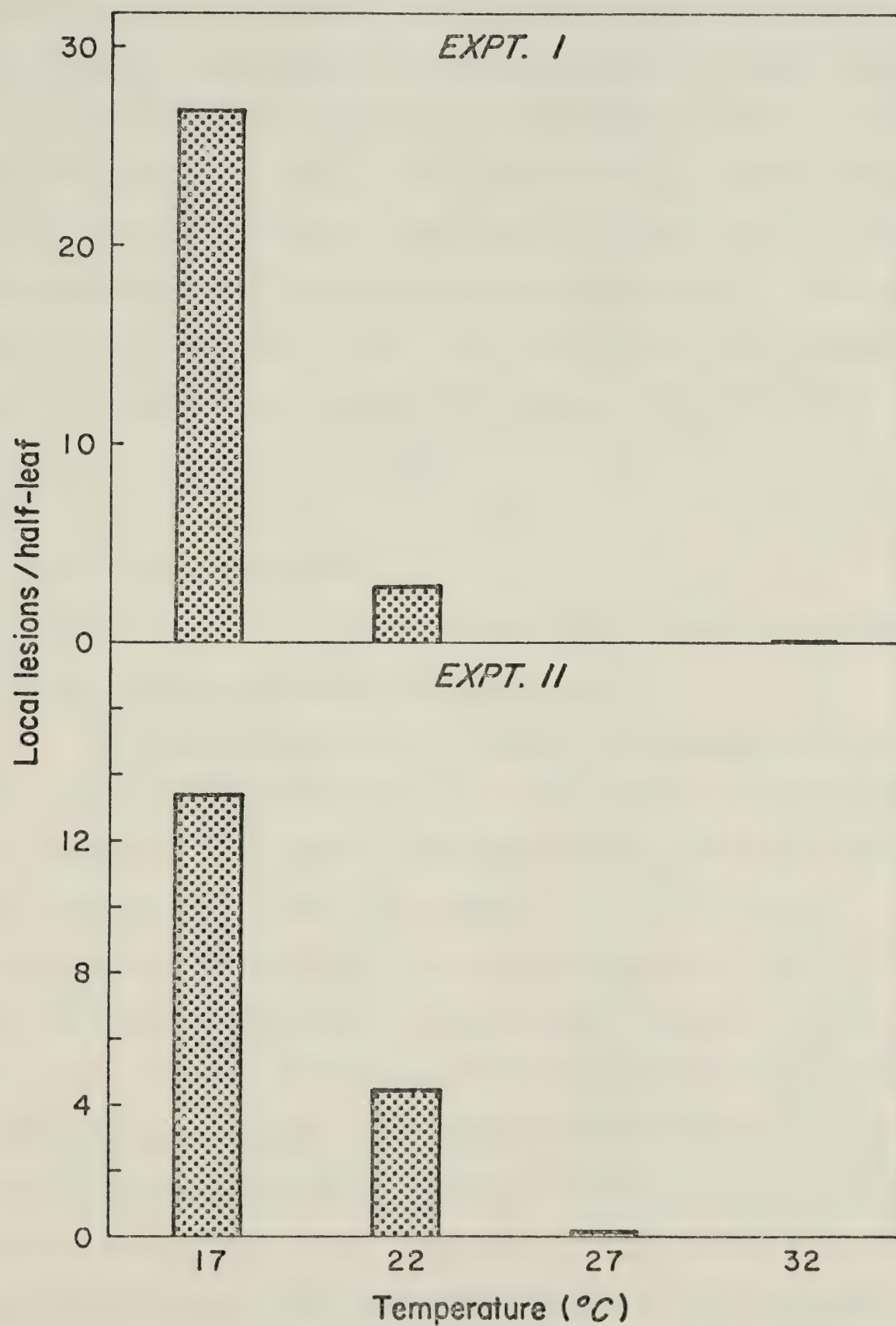
the whole plant is essential to obtain increased susceptibility. The results from the 24-hr dark treatment of *C. quinoa* essentially confirmed those of Hiruki's (1975a) although the 48-hr dark treatment in this investigation resulted in even higher susceptibility. It has been reported that the longer the test plants remained in the darkness (up to 6 days), the more susceptible they became to the subsequent virus infection (Bawden and Roberts, 1948; Costa and Bennett, 1955; Kimmins, 1967).

The inhibitory effect of photosynthetic products on virus multiplication was suggested initially (Bawden and Roberts, 1947, 1948; Yarwood, 1952; Wiltshire, 1956). However, when the inhibitory effect of untreated plant sap was tested against the sap from the dark-treated plant no significant difference was obtained (Bawden and Roberts, 1948). Kimmins (1967) and Kimmins and Litz (1967) found that pre-inoculation treatments in high humidity and darkness increased susceptibility, but when the relative humidity was reduced to 20%, the magnitude of the increase lessened in both darkened and illuminated plants. Therefore, these authors hypothesized that a greater number of infectible sites were rendered available in darkened plants by the increase of leaf turgor pressure.

B. Post-inoculation Temperature

In two separate experiments RKB plants inoculated with PVM showed consistently much higher local lesion number at 17°C (Fig. 6), while at or over 27°C the number of lesions drastically decreased. Using the same local lesion host, Kowalska and Waś (1976) reported that in the temperature range between 16° to 22°C PVM lesions developed

FIG. 6. The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean.



best, but not at 28°C. Using *D. metel* Bagnall et al. (1956b) found that the temperatures between 18° to 20°C were most suitable for the development of local and systemic spot symptoms incited by PVM. Even in potato plants symptoms of PVM infection was reportedly most pronounced at 16°C (Chrzanowska, 1973; Dziewońska and Ostrowska, 1973).

Post-inoculation temperature has its effects both directly on the virus and/or indirectly on the susceptibility of the host plant. Raising the temperature beyond the TIP of virus may increase the chances that particles become inactivated before they can start to reproduce in the host cells (Harrison, 1956). Frost and Harrison (1967) suggested that virus inactivating systems of the host were stimulated more efficiently at a higher temperature.

C. Post Storage Temperature

a. *Infectivity of PVM and PVS extracted from infected potato leaves after storage at different temperatures*

The highest infectivity was obtained from the constantly frozen leaf samples of PVM and PVS (Fig. 7). These results correlated well with Stanley's (1940) similar experiment in which at a 1:10 dilution of sap containing tomato bushy stunt virus a 1.6-fold increase in infectivity was obtained from the sample that had been frozen at -12°C over the fresh control sample. In the present virus-host systems, 1.3- to 1.4-fold increases in infectivity were obtained (Table 3). Harrison (1956) also found that the frozen sample of TNV-infected leaves gave higher infectivity than the fresh one or the sample that had been kept at 22° or at 30°C for 3 days. Therefore, freezing infected leaves, or sap from such leaves have little effect on some viruses (Matthews,

FIG. 7. Infectivity of potato virus M (PVM) and potato virus S (PVS), extracted from infected potato leaves, in 'Red Kidney' bean and *Chenopodium quinoa*, respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls.

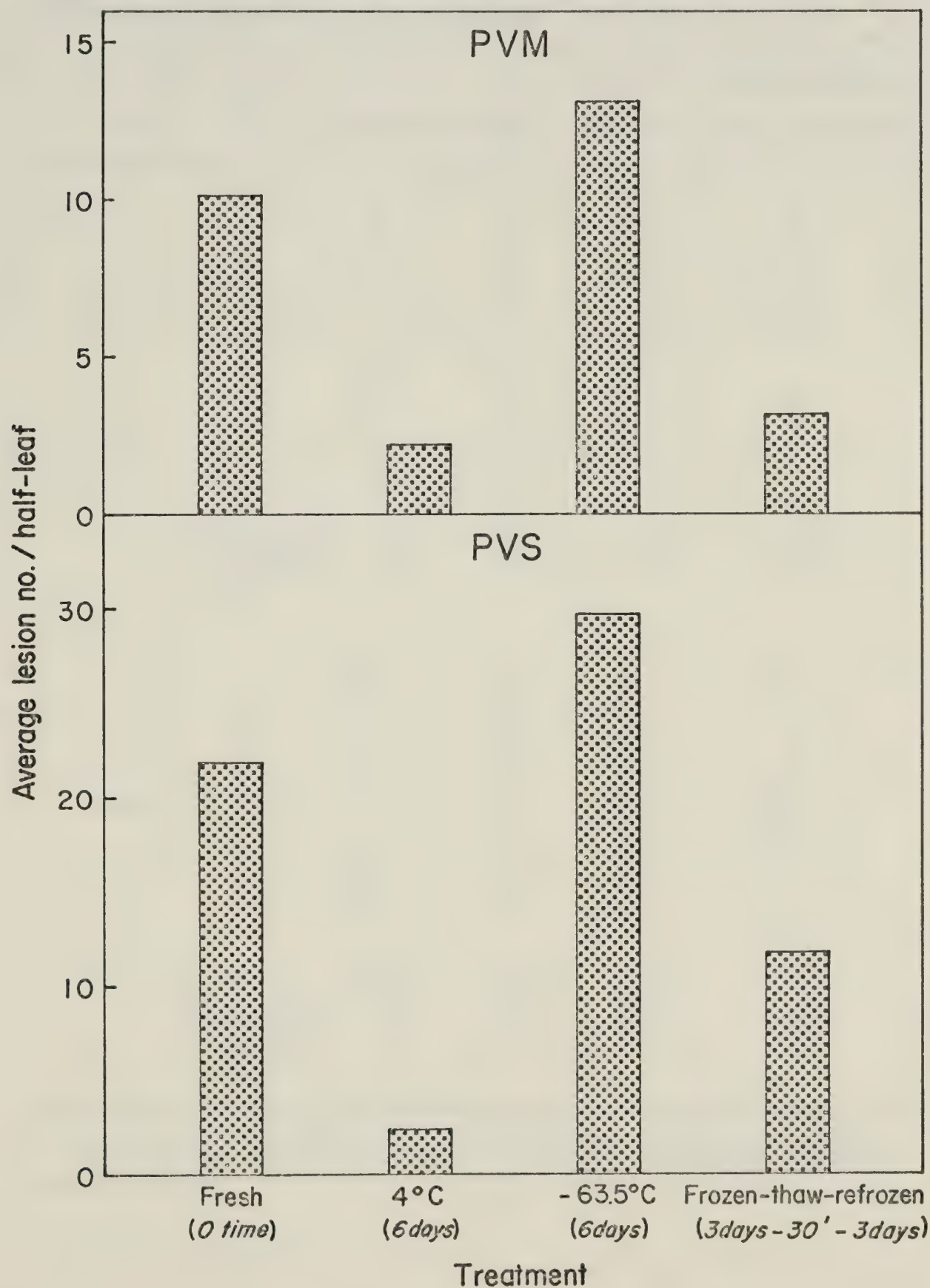


TABLE 3. Lesion counts: Infectivity of potato virus M and potato virus S, extracted from infected potato leaves, in 'Red Kidney' bean (RKB) and *Chenopodium quinoa*, respectively, after storage at different temperatures. Infected fresh leaves from the same plants were used as controls.

Local lesion host ^a	Half- leaf no.	Fresh sap (0 time)	4°C (6 days)	-63.5°C (6 days)	Frozen-thaw-refrozen (3 days-30'-3 days)
R K B	1	9	2	12	2
	2	7	0	5	1
	3	17	6	20	6
	4	4	0	6	1
	5	5	1	6	1
	6	14	5	15	4
	7	10	2	19	5
	8	15	2	22	5
	Mean	10.1	2.3	13.1	3.1
	Standard error	1.7	0.8	2.4	0.7
<i>C. quinoa</i>	Infectivity index	1.0	0.2	1.3	0.3
	1	25	3	32	14
	2	14	0	10	6
	3	15	0	25	4
	4	34	4	45	20
	5	17	2	31	10
	6	19	2	32	12
	7	35	6	32	17
	8	16	2	31	11
	Mean	21.9	2.4	29.8	11.8
	Standard error	3.0	0.7	3.4	1.9
	Infectivity index	1.0	0.1	1.4	0.5

^a Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

1970).

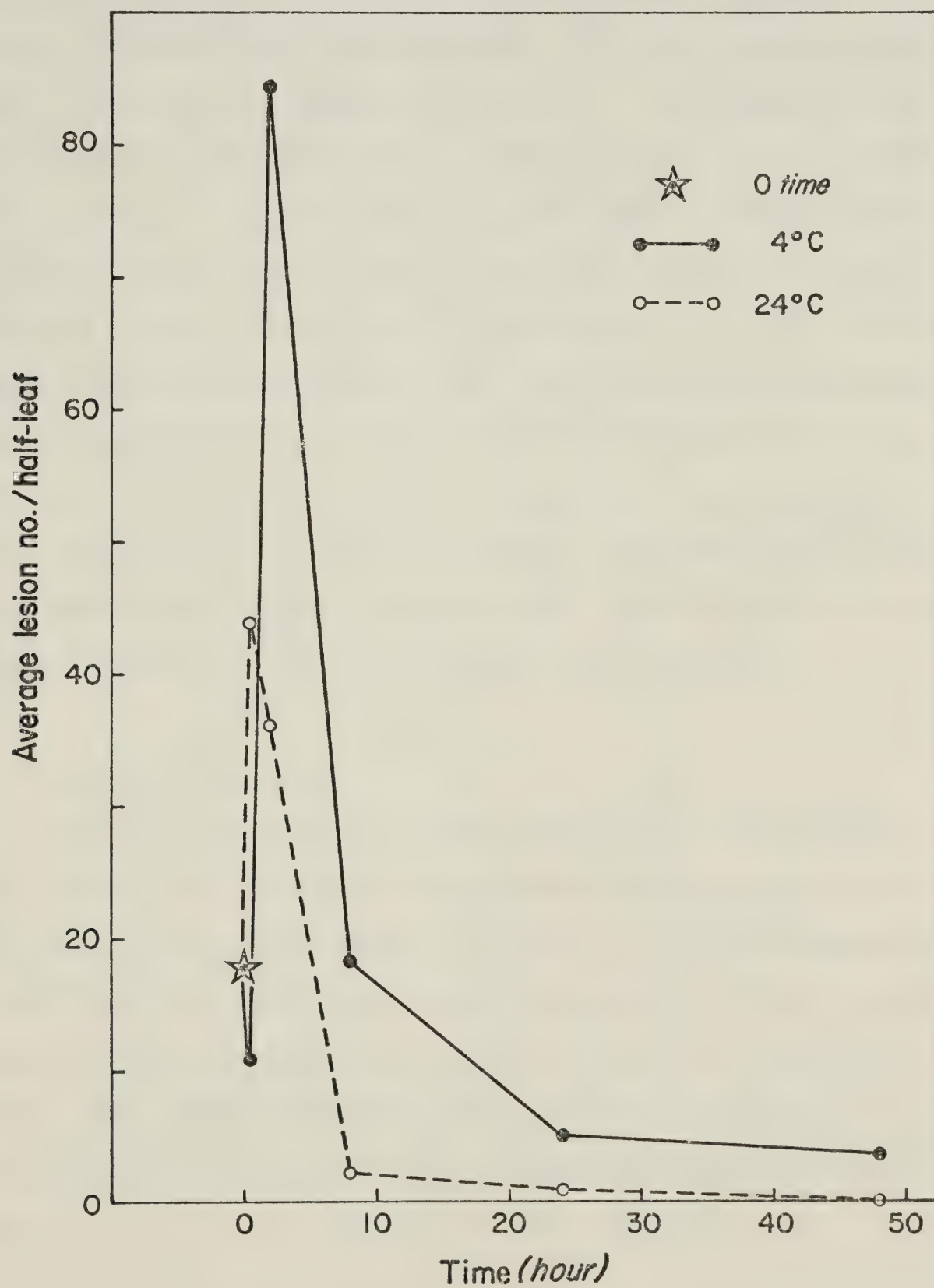
A plausible explanation for the higher infectivity obtained from the frozen samples could be that when the leaves thaw the virus particles are more readily released after the complete disorganization of the cells and that the oxidative enzymes are still in a less active state at such low temperatures. While temperatures near -20°C were claimed to be satisfactory for short term storage (c. 1 year) of some labile viruses (McKinney and Silber, 1968), Meryman (1956) found that all chemical denaturation processes would only appear to stop near -100°C .

The infectivity of the samples subjected to the frozen-thaw-refrozen treatment was lower than that of the fresh ones for both PVM and PVS (Fig. 7). However, the 30-min thawing of the frozen samples at room temperature was insufficient for virus inactivation.

b. Infectivity of PVM extracted from frozen infected potato leaves at various time intervals after incubation at 4° or at 24°C

At zero time the local lesion number obtained was relatively low. PVM extracted from potato leaves that had been frozen and then incubated for 2 and 0.5 hr at 4° and at 24°C respectively, produced the highest number of local lesions (Fig. 8). Thereafter the lesion number steadily decreased up to 48 hr of incubation. Fulton (1957) reported that 4 *Prunus* virus isolates lost their infectivity rapidly in cucurbit sap at 24°C but this loss was slower at 0°C . Prune dwarf, sour cherry recurrent necrotic ringspot and necrotic ringspot viruses also were found losing their infectivity in sap after few hours at room temperature (Hampton and Fulton, 1961).

FIG. 8. Infectivity of potato virus M extracted from frozen infected potato leaves at various time intervals after their incubation at 4° or at 24° C.



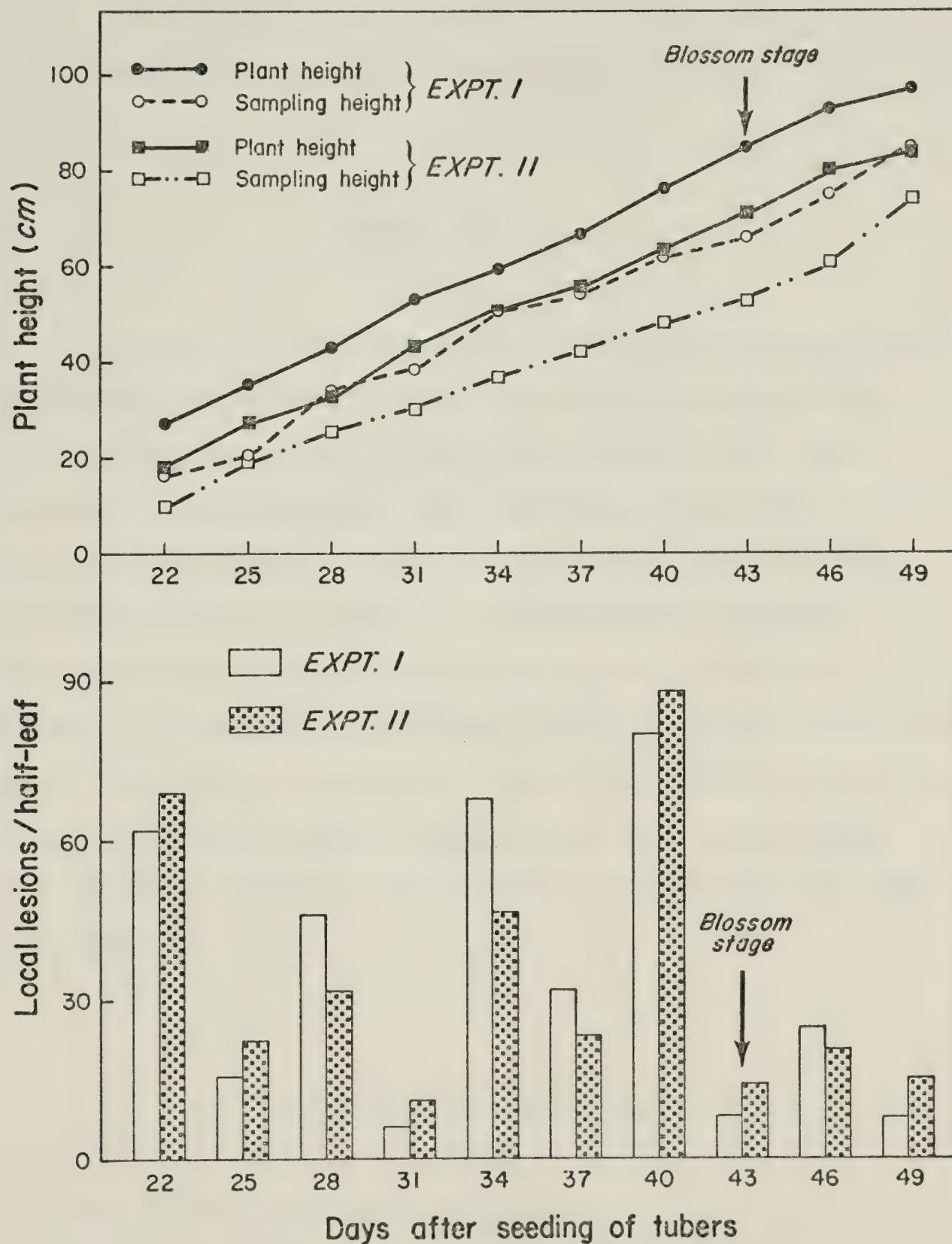
D. Assay of PVM Infectivity at Different Growth Stages of Infected Potato Plants

In both experiments, the relative concentration of PVM estimated by bioassay was initially high, then fluctuated until it reached its maxima just before the blossom stage (Fig. 9). Virus concentration remained low after the appearance of blossoms. The concentration of PVM in infected 'King Edward' potato plants reached a maximum 40 days after seeding. This result agrees with Chrzanowska's (1973) in which serological detection of PVM was best with sap similarly obtained from the upper 2nd, 3rd and 4th leaves of plants aged 5 to 7 weeks. Bartels (1966) also found by serological tests that infected potato and tomato plants showed that PVM was evenly distributed throughout the foliage initially, but after 5 and 8 weeks, respectively, it was generally found only in the upper parts of the plants. Similarly, Wetter (1957) found that with most potato varieties tested, the PVS concentration was highest in leaves just below the growing tip of the shoots.

E. Reciprocal Inoculation

PVM was not recovered from PVM-inoculated *C. quinoa* leaves to RKB; nor could PVM particles be demonstrated by electron microscopy using the same inoculated samples. Similarly, PVS was not detected from the RKB inoculated with PVS by using the same methods. These results agreed with earlier observations (Hiruki, 1970; Hiruki, unpublished data). Thus, taking advantage of this system the interaction of these two viruses could be studied in their respective local lesion hosts.

FIG. 9. Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants.



CHAPTER V

INTERACTION BETWEEN PVM AND PVS IN LOCAL LESION HOST

INTRODUCTION

Although PVM and PVS coexisted in 'King Edward' potatoes, their intermediate types were never found, and this result suggested the lack of genetic recombination between them (Kassanis, 1963). The biological interaction between these two viruses, nevertheless, still remain to be understood. As seen in the foregoing literature review, the use of sap as virus inoculum in virus interaction studies may inevitably result in some undesirable interference between host inhibitors and the interacting viruses, leading to unclear, inconclusive results. Therefore, in the present study purified preparations of PVM, PVS, and their RNA and protein components were used to investigate their biological interactions quantitatively in their respective local lesion hosts.

MATERIALS AND METHODS

A. Interaction Between Intact PVM and PVS

a. Simultaneous inoculation

Purified PVM and PVS were mixed in ratios of 1:1, 1:5 and 1:10 respectively, and the volume of each mixed-virus inoculum was kept constant. The inocula containing PVM and PVS, paired with the inoculum containing PVM only as a control, were applied to RKB. The same treatments, except in the reverse order, were also carried out in *C. quinoa*.

b. Pre- and post-inoculations

In pre-inoculation treatments, inoculation with PVM was carried out using 12 half-leaves of RKB per treatment, and was followed by the secondary inoculations with PVS at 0-, 1-, 5-, and 10-hr intervals respectively. In post-inoculation treatments, inoculation in the reverse order was made also using RKB at the same time. Inoculum containing PVM and phosphate buffer, replacing the same volume of PVS, was applied as a control on the opposite half-leaves. Phosphate buffer alone was also applied to the control half-leaves at the same time when the second virus was applied at the scheduled time intervals. Daily counts of local lesions were made at the fixed time as soon as they were visible. The increment curves were obtained from the local lesion counts for each treatment. The same procedures were used in assaying the infectivity of PVS in the presence of PVM in *C. quinoa*.

c. Simultaneous separate inoculations of upper and lower epidermal layers

Inoculation with PVM was made to the upper epidermis of 12 whole leaves from 12 RKB plants. The lower epidermis of half-leaves was inoculated with PVS and the remaining half-leaves with phosphate buffer. Inoculations were made in the reverse order to the other 12 leaves.

Similar treatments, in the reverse sequence, were repeated in *C. quinoa*.

B. Interaction Between Intact Virus, Viral Ribonucleic Acids (RNA's), Yeast-RNA and Coat-protein

a. *Isolation of viral RNA's and coat-proteins*

The viral RNA's were isolated by the phenol method (Fraenkel-Conrat et al., 1961). The UV-absorption spectrum and the infectivity of each viral RNA preparation were determined. The coat-proteins were isolated by the acetic acid method (Fraenkel-Conrat, 1957) and the resulting preparations were free from intact virus particles when subjected to electron microscopic examinations. The coat-proteins were dissolved in 0.1 N sodium hydroxide, adjusted to pH 8.0, and stored in a deep freezer until use.

b. *Interaction Between intact virus and viral RNA of the counterpart*

A fixed amount of PVM was added to two concentrations of PVS-RNA and applied separately to RKB plants. The same concentration of PVM was used as a control. Inoculum containing PVS and PVM-RNA was similarly tested in *C. quinoa*, with a comparable control containing PVS only.

c. *Simultaneous inoculation with PVM- and PVS-RNA's*

A fixed amount of PVM-RNA was mixed separately with two samples containing different amounts of PVS-RNA for bioassay in RKB, using PVM-RNA only as a control on the opposite half-leaf. Similar treatments were also carried out in the reverse order in *C. quinoa*.

d. *Simultaneous inoculation with intact virus and viral coat-protein of the counterpart*

A fixed amount of PVM was mixed with two preparations containing different amounts of PVS coat-protein and the mixtures were applied separately to RKB plants. Inoculum containing PVM only was applied as a control. The same procedures were applied to *C. quinoa* using inocula containing PVS and PVM coat-protein as tests and PVS only as a control.

e. Detection of endogenous ribonuclease (RNase) activity

A fixed amount of PVM-RNA was mixed with a series of 10-fold dilutions of PVS using phosphate buffer. Five inocula containing PVM and PVS in different ratios were prepared and were applied separately to RKB plants. Unmixed PVM-RNA inoculum was used as a control. The same treatments were carried out in *C. quinoa* with PVS-RNA plus PVM as test inoculum and PVS-RNA as a control.

f. Effect of endogenous RNase on PVS-RNA infectivity in the presence of yeast-RNA

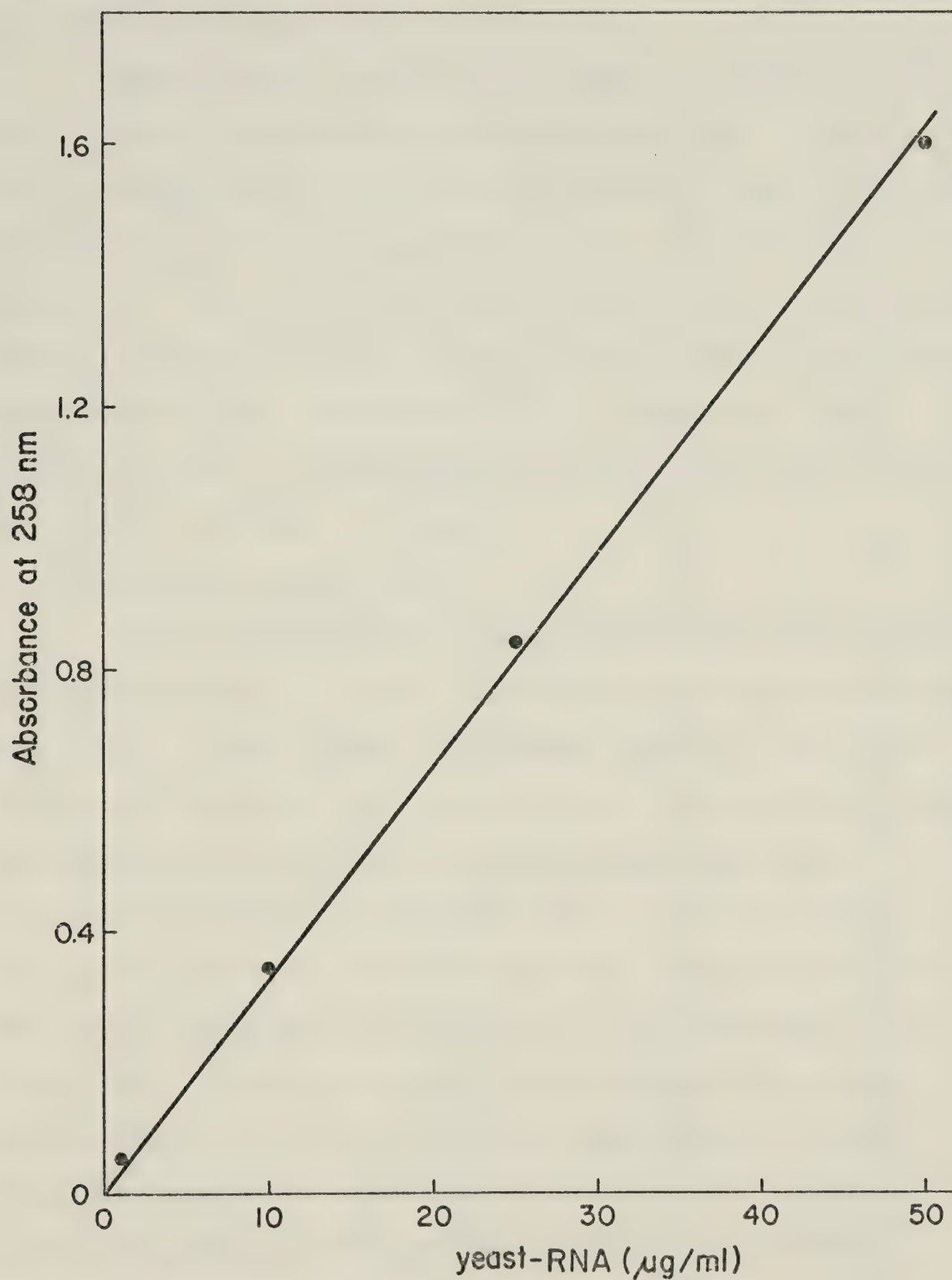
A standard concentration curve of yeast-RNA (Calbiochem, Los Angeles) was obtained by determining absorbance of solutions containing different amount of yeast RNA at 258 nm (Fig. 10). Fixed amounts of PVS-RNA and PVM were mixed with phosphate buffer and two different concentrations of yeast-RNA respectively. The three mixed-inocula were applied separately to *C. quinoa*, while PVS-RNA at the same concentration was applied to the opposite half-leaf as a control. The experiment was repeated with a higher concentration of PVS-RNA.

g. Stimulatory index

In the present study the following formula was used to obtain a stimulatory index from local lesion counts.

$$\text{A stimulatory index} = \frac{\text{Average number of local lesion obtained in test}}{\text{Average number of local lesion obtained in control}}$$

FIG. 10. A Standard curve showing the absorbance values of yeast-ribonucleic acid (RNA) of different concentrations determined at 258 nm.



RESULTS

A. Interaction Between Intact PVM and PVS

a. *Simultaneous inoculation*

Marked increases in local lesion number were obtained in RKB and *C. quinoa* in the presence of the counterpart (Figs. 11 and 12). In RKB the increase was more prominent as the amount of added PVS increased and the stimulatory index ranged from 1.4 to 7.1 (Tables 4A and 4B). However, in *C. quinoa* the lesion numbers incited by inocula containing PVM and PVS in the ratios of 1:5 and 1:10 did not differ significantly. The stimulatory index ranged from 1.4 to 2.1 for PVS in *C. quinoa* (Tables 5A and 5B). In application of phosphate buffer only, no lesion was incited in both hosts as expected.

b. *Pre- and post-inoculations*

At zero time immediate, sequential inoculations were made with PVM and PVS separately. Similar inoculations in the reverse order were made as well. These treatments consistently resulted in the highest infectivity of the test virus, and the extent of the stimulatory effect was comparable to that obtained from simultaneous inoculation experiments described in the foregoing section. The pre or post-inoculations with PVM or PVS at different time intervals resulted in the limited stimulation of the infectivity of the counterpart (Figs. 13A and 13B). In general, the post-inoculation experiments showed slightly higher stimulatory indexes than those obtained in the pre-inoculation experiments in both hosts (Table 6.). The stimulatory indexes decreased as the time interval between the pre- and post-inoculations was prolonged.

FIG. 11. Increases in the local lesion number of potato virus M obtained by applying a mixed inoculum of purified potato viruses M and S in different ratios to 'Red Kidney' bean.

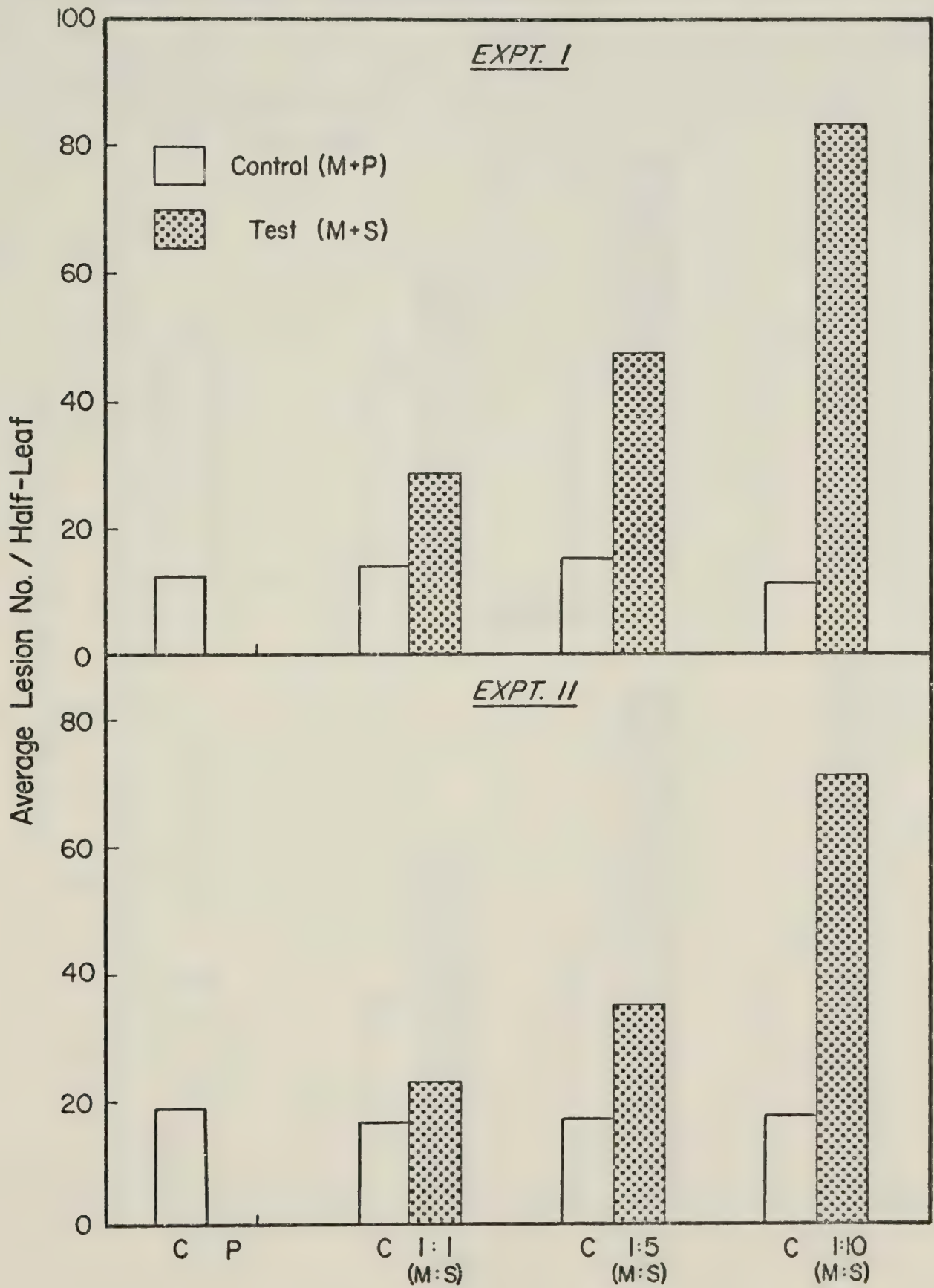


FIG. 12. Increases in the local lesion number of potato virus S obtained by applying a mixed inoculum of purified potato viruses S and M in different ratios to *Chenopodium quinoa*.

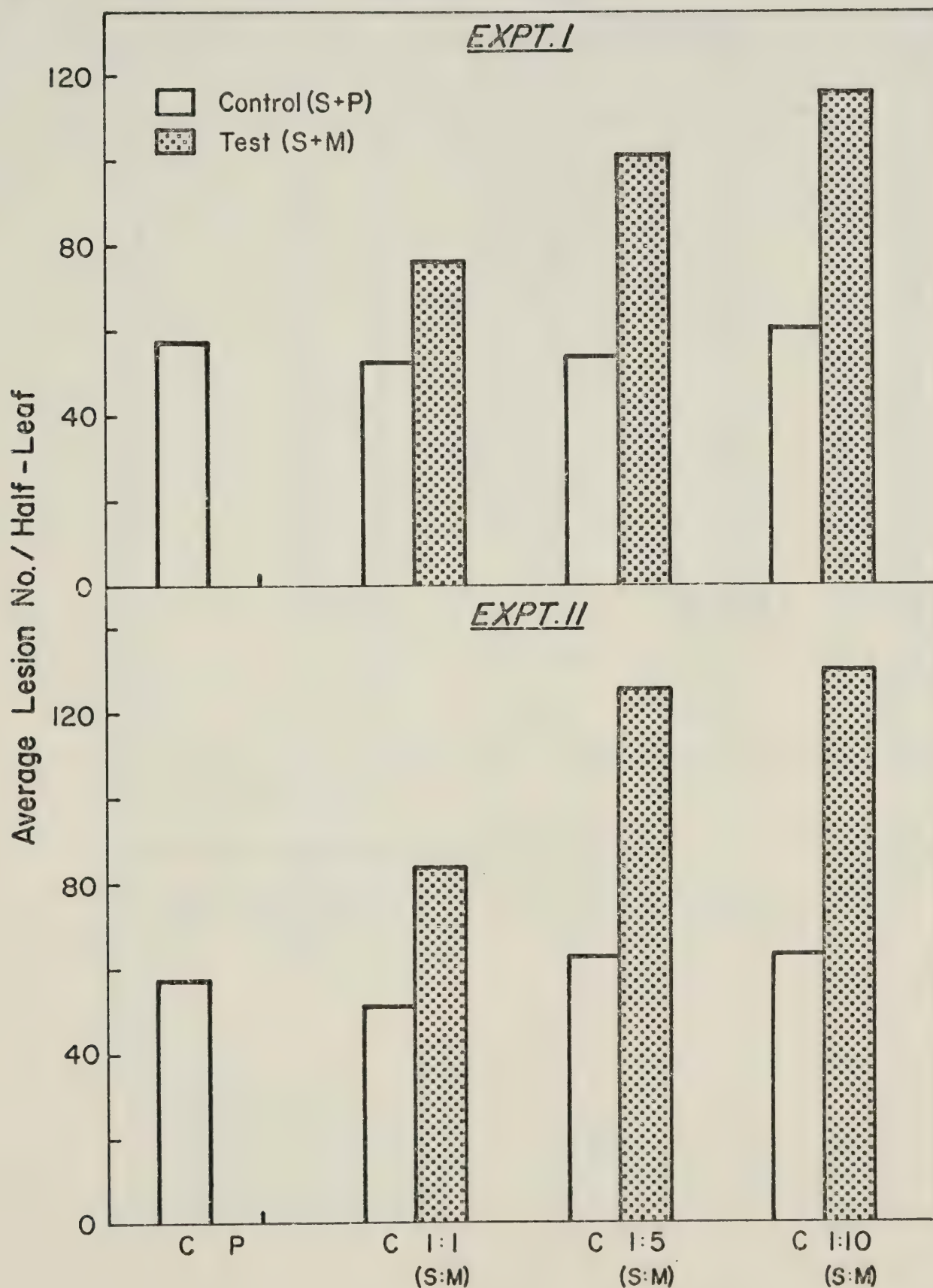


TABLE 4 A. The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. *Expt. I.*

Half-leaf no.	Lesion no. / half-leaf							
	C ^a	P ^b	C	1:1 ^c	C	1:5 ^c	C	1:10 ^c
1	8	0	12	20	20	39	12	81
2	13	0	21	19	17	30	7	54
3	22	0	6	24	15	33	23	96
4	11	0	11	30	13	48	16	67
5	5	0	13	32	12	23	10	45
6	13	0	14	17	21	28	6	73
7	11	0	7	36	19	79	7	64
8	7	0	10	50	6	59	11	85
9	12	0	21	15	28	75	9	133
10	6	0	26	11	12	57	13	101
11	12	0	12	54	9	21	25	110
12	15	0	24	38	11	57	12	96
13	27	0	9	23	13	64	8	69
14	10	0	11	32	20	35	5	97
Mean	12.3	0	14.1	28.6	15.4	46.3	11.7	83.6
Standard error	1.6	0	1.7	3.4	1.5	5.1	1.6	6.3
Stimulatory index			2.0		3.1		7.1	

^a C = control, PVM only.

^b P = 0.057 M phosphate buffer (pH 8.0) only.

^c 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS ; 1:10 = 1 part PVM + 10 parts PVS.

TABLE 4B. The local lesion numbers obtained from simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) mixed in different ratios to 'Red Kidney' bean. *Expt. II.*

Half-leaf no.	Lesion no. / half-leaf							
	C ^a	P ^b	C	1:1 ^c	C	1:5 ^c	C	1:10 ^c
1	16	0	13	11	12	24	13	97
2	8	0	20	12	9	31	32	128
3	30	0	12	17	27	48	25	101
4	23	0	12	29	17	39	25	125
5	16	0	18	33	13	34	14	78
6	16	0	25	24	8	25	22	78
7	23	0	14	15	21	36	8	61
8	13	0	10	11	11	41	17	45
9	29	0	16	34	19	43	15	39
10	10	0	12	57	22	32	11	31
11	13	0	17	12	35	44	9	35
12	11	0	27	17	24	32	15	49
13	18	0	22	23	22	24	18	44
14	14	0	14	11	16	41	21	89
15	19	0	10	39	9	34	14	98
16	31	0	13	34	12	48	10	52
17	18	0	15	37	13	10	30	79
18	27	0	15	13	10	18	23	78
19	13	0	21	19	25	37	17	100
20	19	0	16	17	12	23	16	54
21	15	0	21	19	23	37	24	75
22	21	0	13	26	14	14	10	68
23	33	0	20	17	23	45	12	54
24	21	0	16	27	12	76	16	52
Mean	19.0	0	16.3	23.1	17.0	34.8	17.4	71.3
Standard error	1.4	0	0.9	2.3	1.4	2.8	1.3	5.6
Stimulatory index			1.4		2.1		4.1	

^a C=control, PVM only.

^b P=0.057 M phosphate buffer (pH 8.0) only.

^c 1:1= 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS ; 1:10= 1 part PVM + 10 parts PVS.

TABLE 5A. The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to *Chenopodium quinoa*. *Expt. I.*

Half-leaf no.	Lesion no. / half-leaf							
	C ^a	P ^b	C	1:1 ^c	C	1:5 ^c	C	1:10 ^c
1	44	0	59	71	42	109	58	111
2	63	0	47	57	66	131	47	118
3	53	0	39	52	40	83	63	140
4	75	0	31	53	33	99	53	103
5	29	0	30	48	39	64	47	109
6	39	0	27	44	41	82	68	129
7	45	0	31	67	50	83	84	134
8	68	0	53	86	46	97	104	174
9	31	0	83	102	22	74	37	70
10	44	0	119	142	27	89	47	87
11	69	0	86	117	40	106	70	143
12	94	0	73	124	30	138	90	161
13	40	0	41	67	40	67	30	75
14	54	0	58	89	43	71	41	85
15	83	0	45	64	91	118	53	74
16	59	0	32	53	66	92	50	87
17	33	0	44	61	46	72	40	74
18	67	0	75	119	76	95	38	57
19	78	0	80	106	75	86	58	96
20	104	0	73	93	83	109	66	103
21	37	0	30	47	62	111	68	119
22	57	0	33	35	78	138	51	144
23	50	0	36	56	93	150	90	171
24	73	0	43	76	69	176	106	219
Mean	57.9	0	52.8	76.2	54.1	101.7	60.8	116.0
Standard error	4.1	0	4.8	6.0	4.3	5.8	4.3	8.1
Stimulatory index			1.4		1.9		1.9	

^a C = control, PVM only.

^b P = 0.057 M phosphate buffer (pH 8.0) only.

^c 1:1 = 1 part PVM + 1 part PVS ; 1:5 = 1 part PVM + 5 parts PVS;
1:10 = 1 part PVM + 10 parts PVS.

TABLE 5 B. The local lesion numbers obtained from simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) mixed in different ratios to *Chenopodium quinoa*. Expt. II.

Half-leaf no.	Lesion no./half-leaf							
	C ^a	P ^b	C	1:1 ^c	C	1:5 ^c	C	1:10 ^c
1	38	0	45	56	89	126	76	81
2	51	0	38	92	115	225	84	94
3	43	0	51	96	122	135	70	86
4	33	0	53	67	103	129	72	72
5	32	0	30	52	70	104	73	104
6	37	0	48	46	81	157	81	258
7	42	0	42	65	74	238	55	180
8	29	0	34	39	49	103	47	156
9	36	0	86	81	35	119	111	103
10	50	0	100	158	44	129	126	145
11	61	0	72	129	53	139	95	233
12	28	0	60	117	40	98	92	129
13	100	0	45	79	80	108	50	139
14	103	0	72	100	103	141	65	101
15	100	0	93	122	71	160	48	158
16	94	0	55	83	48	148	40	69
17	88	0	25	94	39	64	39	125
18	111	0	28	103	40	86	45	137
19	99	0	33	110	38	100	46	102
20	64	0	30	112	32	87	33	137
21	34	0	42	45	30	111	34	180
22	50	0	44	41	41	129	43	131
23	27	0	55	65	50	105	47	123
24	29	0	45	54	54	76	43	78
Mean	57.5	0	51.1	83.6	62.5	125.7	63.1	130.0
Standard error	6.0	0	4.2	6.5	5.7	8.3	5.1	9.7
Stimulatory index			1.6		2.0		2.1	

^a C = control, PVM only.

^b P = 0.057 M phosphate buffer (pH 8.0) only.

^c 1:1 = 1 part PVM + 1 part PVS; 1:5 = 1 part PVM + 5 parts PVS;
1:10 = 1 part PVM + 10 parts PVS.

FIG. 13A. Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or *Chenopodium quinoa*. Expt. 1.

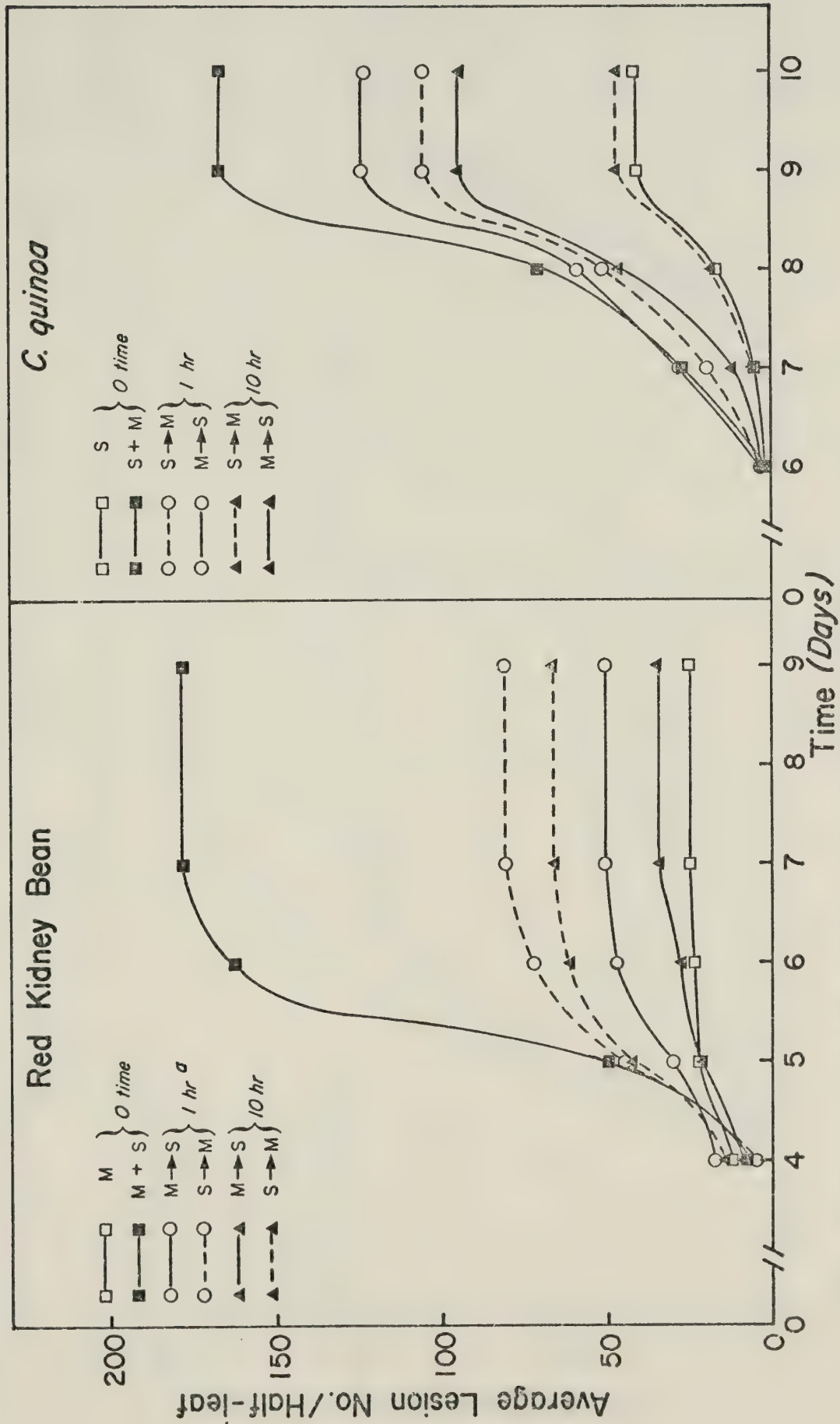


FIG. 13B. Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean or *Chenopodium quinoa*. Expt. 11.

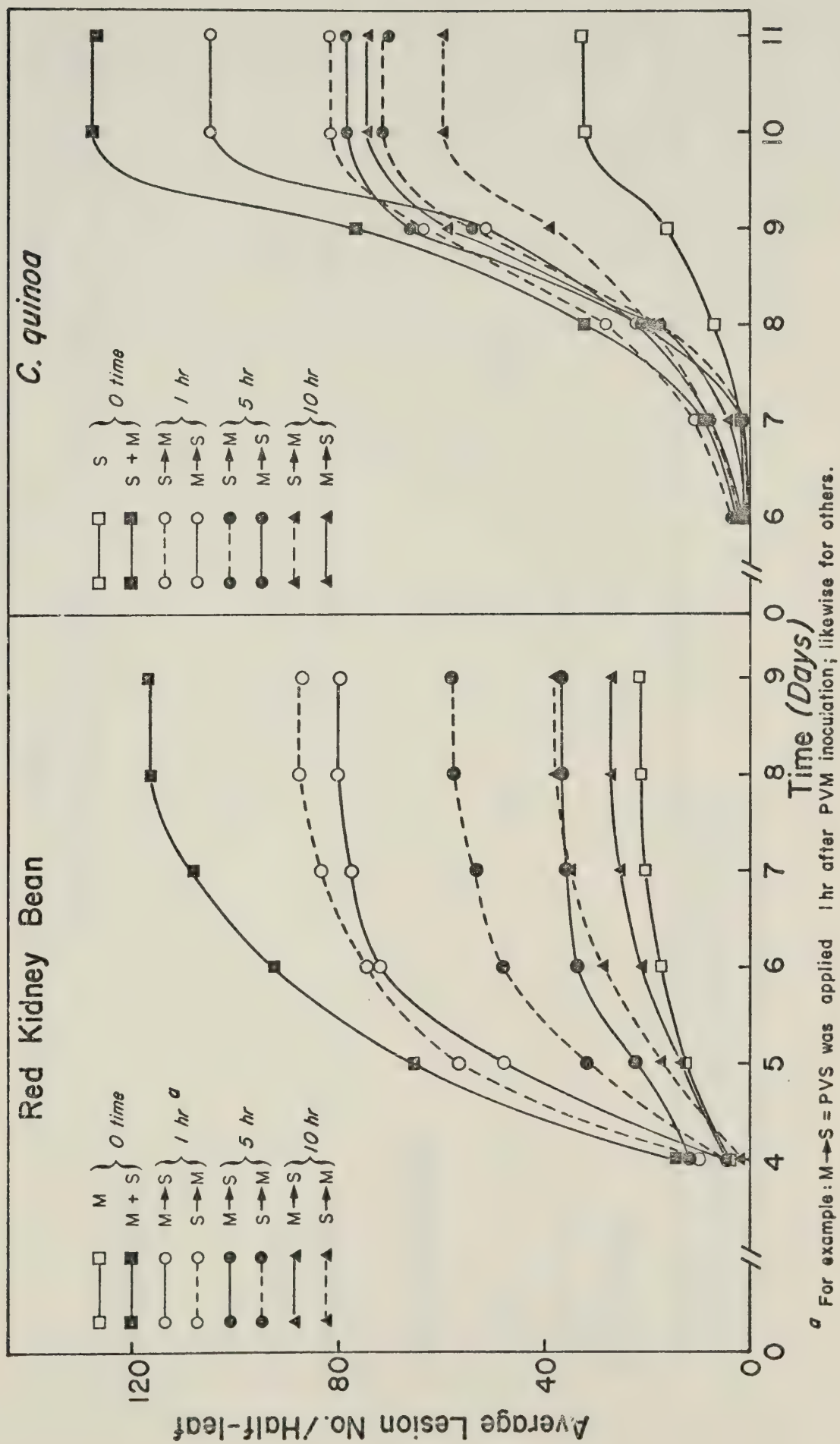


TABLE 6. Stimulatory indexes obtained from the pre- and post-inoculation treatments with purified potato virus M(PVM) and potato virus S(PVS) in 'Red Kidney' bean or *Chenopodium quinoa*.

	Stimulatory Index									
	EXPT. I				EXPT. II					
	Control	O time	1 hr	10 hrs	Control	O time	1 hr	5 hrs	10 hrs	
<u>Inocula in RKB</u>										
M	1.0				1.0					
M + S		7.1				5.4				
M→S (Pre) ^a			2.0	1.4			3.7	1.7	1.3	
S→M (Post) ^b			3.2	2.7			4.0	2.7	1.7	
<u>Inocula in C. quinoa</u>										
S	1.0				1.0					
S + M		4.0				4.0				
S→M (Pre)			2.5	1.1			2.5	2.2	1.8	
M→S (Post)			3.0	2.3			3.2	2.4	2.3	

^a PVM inoculation was followed by PVS.

^b PVS inoculation was followed by PVM.

In RKB, PVM lesions from all treatments started to appear 4 days after inoculation, then increased rapidly between 5 and 6 days and reached a maximum in 7 to 8 days (Figs. 13A and 13B). In *C. quinoa*, PVS lesions were visible 6 days after inoculation, then increased logarithmically during the period between 7 to 9 days and reached a maximum in 9 to 10 days (Figs. 13A and 13B).

c. Simultaneous separate inoculations of upper and lower epidermal layers

There was no significant difference in the stimulatory index in simultaneous separate inoculations with PVM and PVS of the upper and lower epidermal layers of RKB and *C. quinoa* leaves (Tables 7 and 8). The results also indicated that the susceptibility of the lower epidermis of both host plants was much lower than that of the upper epidermis.

B. Interaction Between Intact Virus, Viral RNA's, Yeast-RNA and Coat-protein

a. Infectivity of viral RNA's

The UV-absorption spectra of PVM- and PVS-RNA's obtained were typical for RNA (Fig. 14). The ratio of A max./min. for PVM-RNA and PVS-RNA were 2.26 and 2.25 respectively. The infectivity of the PVS-RNA preparation was higher than that of the PVM-RNA (Fig. 15).

b. Interaction between intact virus and viral RNA of the counterpart

Infectivity of the intact virus was decreased in the presence of the viral RNA of the counterpart (Tables 9 and 10). The results showed that the degree of interference was dependent on the concentration of the viral RNA of the counterpart present in the inoculum.

TABLE 7. Simultaneous separate inoculations with purified potato virus M(PVM) and potato virus S (PVS) of the upper and lower epidermal layers of 'Red Kidney' bean.

Expt.	Leaf no.	Lesion no./half-leaf							
		M ^a		M		P S		S P	
		P ^b	S ^c	S	P	P	M	S	M
I	1	30	14	26	27	6	4	7	4
	2	38	32	46	50	9	7	7	9
	3	18	29	13	10	2	5	6	6
	4	37	31	17	29	10	13	10	9
	5	18	11	19	24	4	8	5	4
	6	46	40	29	31	2	6	9	10
	7	15	18	16	25	3	4	3	3
	8	34	43	31	44	8	11	15	11
	9	25	26	19	22	10	12	12	10
	10	29	25	33	41	7	4	4	5
	11	27	22	16	13	5	2	9	4
	12	17	12	10	21	8	6	8	5
Mean		M/P(Control) = 28.0 M/S(Test) = 24.1				P/M(Control) = 6.4 S/M(Test) = 7.4			
Stimulatory index		Test/Control = 0.9				Test/Control = 1.2			
II	1	15	10	11	22	2	1	4	3
	2	11	15	19	17	4	4	3	2
	3	20	15	13	24	4	2	5	5
	4	35	40	30	30	3	3	6	4
	5	8	15	14	10	5	6	7	10
	6	19	23	20	14	8	11	13	9
	7	18	12	10	21	10	8	12	7
	8	24	21	27	17	7	6	10	8
	9	41	39	30	36	2	4	6	5
	10	15	17	21	13	4	2	9	6
	11	12	11	19	14	5	8	7	12
	12	28	14	16	19	14	9	10	7
Mean		M/P(Control) = 20.1 M/S(Test) = 19.3				P/M(Control) = 6.1 S/M(Test) = 6.5			
Stimulatory index		Test/Control = 1.0				Test/Control = 1.1			

^a 0.33 mg PVM/ml was applied to the whole upper epidermis.

^b 0.057 M phosphate buffer (pH 8) was applied to one-half of the lower epidermis.

^c 0.40 mg PVS/ml was applied to one-half of the lower epidermis. Similar scheme applies for other inoculations.

TABLE 8. Simultaneous separate inoculations with purified potato virus S(PVS) and potato virus M (PVM) of the upper and lower epidermal layers of *Chenopodium quinoa*.

Expt.	Leaf no.	Lesion no./half-leaf							
		S ^a		S		P M		M P	
		P ^b	M ^c	M	P	S		S	
I	1	31	33	50	44	23	28	26	14
	2	41	48	45	38	31	44	53	35
	3	49	67	60	46	15	18	22	21
	4	77	63	59	60	27	40	25	34
	5	70	76	79	63	42	51	35	50
	6	50	59	42	40	22	16	28	18
	7	33	47	32	30	26	23	24	24
	8	60	81	42	49	25	29	23	31
	9	47	30	31	30	32	27	34	20
	10	30	21	25	44	21	25	16	19
	11	23	32	20	25	22	17	15	20
	12	37	30	34	37	24	31	27	25
Mean		S/P(Control) = 43.9				P/S(Control) = 25.9			
		S/M(Test) = 46.1				M/S(Test) = 28.2			
Stimulatory index		Test/Control = 1.1				Test/Control = 1.1			
II	1	21	35	40	30	27	25	31	20
	2	51	37	45	38	30	20	22	17
	3	44	39	54	47	25	26	31	29
	4	30	29	31	42	45	52	47	34
	5	29	27	36	44	37	40	37	41
	6	64	60	70	89	49	60	59	53
	7	70	54	66	51	19	30	25	26
	8	49	39	63	62	32	18	39	26
	9	80	95	80	93	35	38	45	28
	10	21	26	26	33	20	26	38	26
	11	53	47	50	72	45	70	56	61
	12	35	33	46	70	22	33	20	37
Mean		S/P(Control) = 50.8				P/S(Control) = 32.7			
		S/M(Test) = 47.0				M/S(Test) = 37.0			
Stimulatory index		Test/Control = 0.9				Test/Control = 1.1			

^a 0.16 mg of PVS/ml was applied to the whole upper epidermis.

^b 0.057 M phosphate buffer (pH 8) was applied to one-half of the lower epidermis.

^c 1.67 mg PVM/ml was applied to one-half of the lower epidermis.

Similar scheme applies for other inoculations.

FIG. 14. The UV spectra of ribonucleic acids (RNA's) respectively isolated from potato virus M(PVM) and potato virus S (PVS).

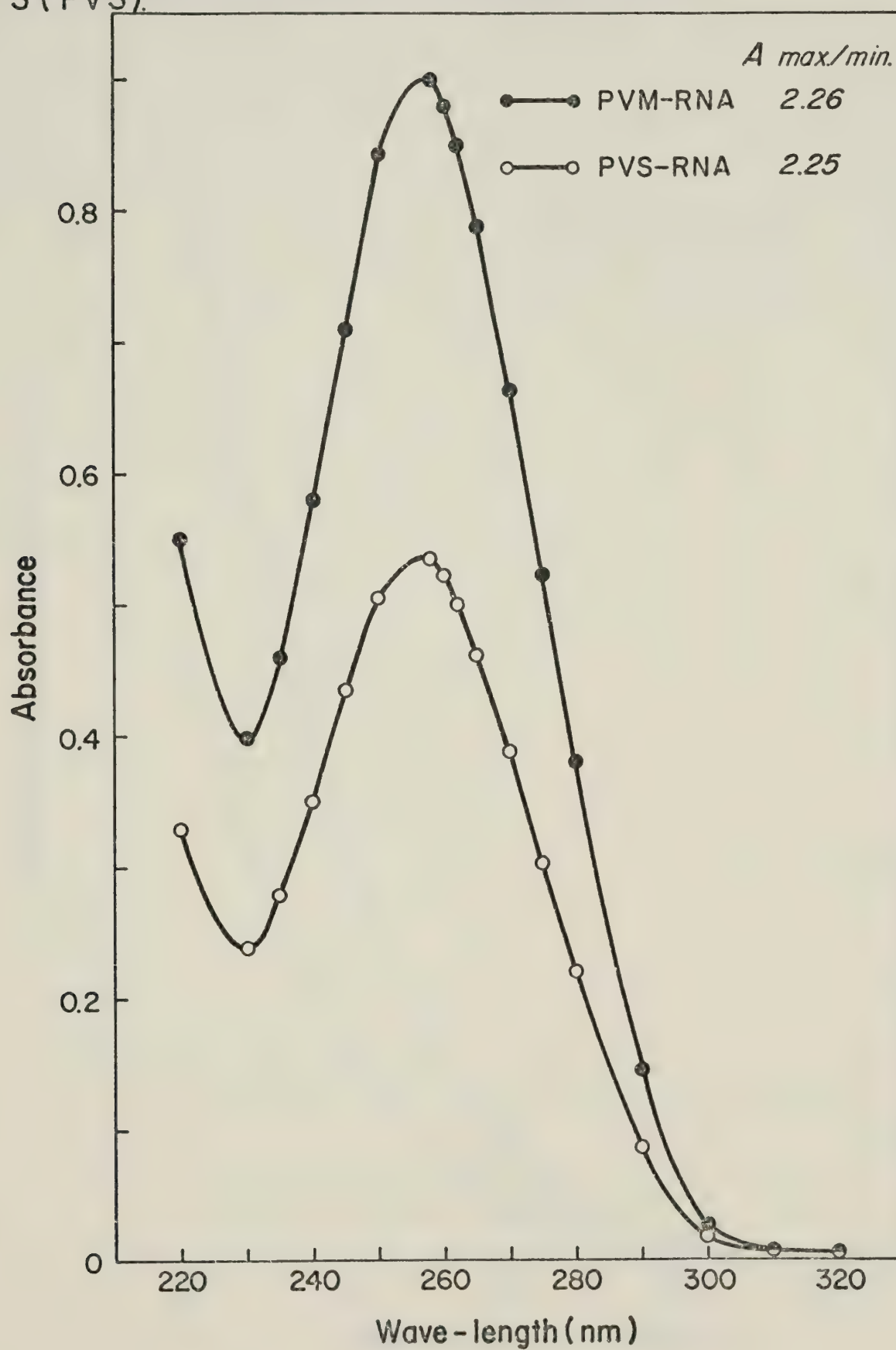


FIG. 15. The dilution curves of the infectivity of ribonucleic acids (RNA's) respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean and *Chenopodium quinoa*.

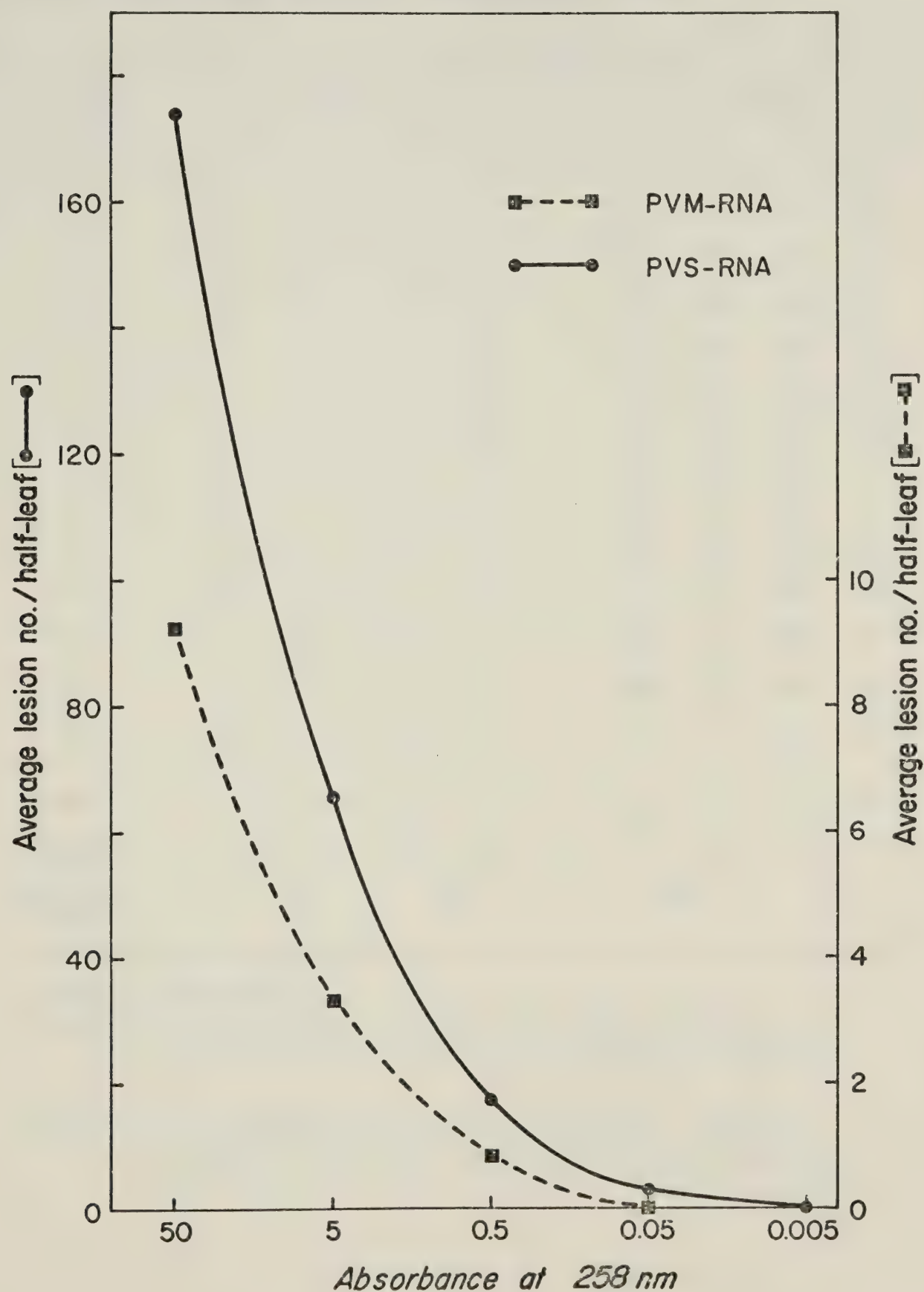


TABLE 9. Interaction between intact potato virus M (PVM) and potato virus S (PVS)-ribonucleic acid (RNA) in 'Red Kidney' bean.

Half-leaf no.	Lesion no. / half-leaf							
	<i>Expt. I</i>				<i>Expt. II</i>			
	<i>C^a</i>	<i>T₁^b</i>	<i>C^a</i>	<i>T₂^b</i>	<i>C^a</i>	<i>T₁^c</i>	<i>C^a</i>	<i>T₂^c</i>
1	50	43	75	30	35	14	70	38
2	81	28	68	35	105	35	92	21
3	62	22	116	67	64	20	51	24
4	61	15	135	82	56	23	80	30
5	79	32	143	62	73	17	68	32
6	100	23	84	43	27	30	60	46
7	79	18	58	71	43	18	62	35
8	75	21	68	52	59	25	42	29
9	81	40	100	46	37	10	50	40
10	71	33	106	44	75	19	45	26
11	61	27	74	27	108	33	36	30
12	111	24	54	26	54	16	120	59
Mean	75.9	27.2	90.1	48.8	61.3	21.7	64.7	34.2
Standard error	4.9	2.5	8.6	5.3	7.4	2.2	6.9	3.0
Stimulatory index	0.4		0.5		0.4		0.5	

^a Control: 1.1 mg/ml PVM.

^b Test (I) : T_1 = 1.1 mg/ml PVM + 5 μ units of PVS-RNA at 258 nm;
 T_2 = 1.1 mg/ml PVM + 0.5 μ units of PVS-RNA at 258 nm.

^c Test (II) : T_1 = 1.1 mg/ml PVM + 6 μ units of PVS-RNA at 258 nm;
 T_2 = 1.1 mg/ml PVM + 0.6 μ units of PVS-RNA at 258 nm.

TABLE 10. Interaction between intact potato virus S (PVS) and potato virus M (PVM)-ribonucleic acid (RNA) in *Chenopodium quinoa*.

Half-leaf no.	Lesion no / half-leaf							
	<i>Expt. I</i>				<i>Expt. II</i>			
	C^a	T_1^b	C^a	T_2^b	C^c	T_1^d	C^c	T_2^d
1	52	11	57	36	36	11	24	10
2	61	45	60	42	77	20	89	58
3	40	20	39	29	45	11	22	25
4	77	31	71	52	29	19	48	12
5	26	26	52	27	50	7	19	33
6	45	30	33	24	39	25	66	13
7	59	40	28	22	50	12	57	14
8	39	14	31	38	48	35	40	22
9	43	23	50	40	37	10	33	43
10	51	33	101	71	28	8	75	17
11	48	24	67	20	43	9	66	30
12	90	13	54	43	25	15	29	29
Mean	52.6	25.8	53.6	37.0	42.3	15.2	47.3	25.5
Standard error	5.0	3.1	5.9	4.2	4.0	2.4	6.7	4.1
Stimulatory index	0.5		0.7		0.4		0.5	

^a Control (I): 0.24 mg/ml PVS.

^b Test (I): T_1 = 0.24 mg/ml PVS + 3.0 μ units of PVM-RNA at 258 nm;
 T_2 = 0.24 mg/ml PVS + 3.0 μ units of PVM-RNA at 258 nm.

^c Control (II): 0.16 mg/ml PVS.

^d Test (II): T_1 = 0.16 mg/ml PVS + 3.0 μ units of PVM-RNA at 258 nm;
 T_2 = 0.16 mg/ml PVS + 3.0 μ units of PVM-RNA at 258 nm.

The results also indicated the reductions in the lesion number due to the presence of the RNA of the counterpart were approximately 50 to 60% for PVM in RKB (Table 9) and 30 to 60% for PVS in *C. quinoa* (Table 10).

c. Simultaneous inoculation with PVM- and PVS-RNA's

Only slight interference was induced by the viral RNA of the counterpart. The results showed that about a 10% inhibition of the PVM-RNA infectivity occurred in RKB (Table 11) and about 20 to 30% inhibition of PVS-RNA infectivity in *C. quinoa* (Table 12).

d. Simultaneous inoculation with intact virus and viral coat-protein of the counterpart

In both cases, the infectivity of the test viruses was slightly higher in the presence of the coat-protein of the counterpart in the inoculum. On the whole, as the stimulatory index indicates, about 10 to 30% increases in local lesion number were obtained (Tables 13 and 14). The inoculum containing coat-protein only did not produce any lesion.

e. Detection of endogeneous RNase activity

In both cases the infectivity of PVM- and PVS-RNA's was higher as the concentration of the intact viral counterpart was reduced (Fig. 16 and 17). The activity of the endogenous RNase apparently diminished upon diluting the preparation containing the intact viral counterpart between the dilutions 10^{-2} to 10^{-4} .

f. Effect of endogenous RNase on PVS-RNA infectivity in the presence of yeast-RNA

The infectivity of PVS-RNA was retained in the presence of yeast-RNA in the inoculum (Table 15). As the concentration of yeast-RNA in the inocula increased, there were apparent decreases in

TABLE 11. Simultaneous application of potato virus M (PVM) - and potato virus S (PVS)-ribonucleic acids (RNA's) to 'Red Kidney' bean.

Half-leaf no.	Lesion no. / half-leaf			
	<i>Expt. I</i>		<i>Expt. II</i>	
	<i>C^a</i>	<i>T^b</i>	<i>C^a</i>	<i>T^c</i>
1	3	4	5	6
2	8	6	6	3
3	18	15	15	14
4	11	10	12	10
5	5	6	6	7
6	4	3	3	3
7	8	5	10	10
8	6	5	4	3
9	10	10	8	7
10	15	12	11	9
11	9	8	12	11
12	11	13	9	9
Mean	9.0	8.1	8.4	7.7
Standard error	1.3	1.1	1.1	1.0
Stimulatory index	0.9		0.9	

^a Control: 40 *A* units of PVM-RNA at 258 nm.

^b Test(I): 40 *A* units of PVM-RNA + 6 *A* units of PVS-RNA, all at 258 nm.

^c Test(II): 40 *A* units of PVM-RNA + 12 *A* units of PVS-RNA, all at 258 nm.

TABLE 12. Simultaneous application of potato virus S (PVS)- and potato virus M (PVM)-ribonucleic acids (RNA's) to *Chenopodium quinoa*.

Half-leaf no.	Lesion no. / half-leaf			
	<i>Expt. I</i>		<i>Expt. II</i>	
	C ^a	T ^b	C ^a	T ^c
1	109	115	129	78
2	59	42	69	45
3	222	200	212	208
4	178	160	208	169
5	119	78	152	92
6	115	88	134	77
7	152	112	175	121
8	144	69	114	64
9	89	51	92	51
10	120	91	149	106
11	169	115	211	122
12	155	99	203	107
Mean	135.9	101.7	154.0	103.3
Standard error	12.6	12.8	14.1	13.8
Stimulatory index	0.8		0.7	

^a Control: 6 A units of PVS-RNA at 258 nm.

^b Test(I): 6 A units of PVS-RNA + 40 A units of PVM-RNA, all at 258 nm.

^c Test(II): 6 A units of PVS-RNA + 30 A units of PVM-RNA, all at 258 nm.

TABLE 13. Simultaneous application of purified potato virus M (PVM) and potato virus S (PVS) coat-protein to 'Red Kidney' bean.

Half-leaf no.	Lesion no. / half-leaf					
	<i>Expt. I</i>			<i>Expt. II</i>		
	<i>C^a</i>	<i>T^b</i>	Coat-protein only ^c	<i>C^a</i>	<i>T^d</i>	Coat-protein only ^c
1	101	118	0	31	30	0
2	39	96	0	72	89	0
3	27	39	0	63	56	0
4	78	51	0	30	47	0
5	41	39	0	23	45	0
6	72	68	0	69	41	0
7	80	106	0	45	40	0
8	66	73	0	74	95	0
9	33	40	0	32	79	0
10	34	66	0	73	78	0
11	41	42	0	40	49	0
12	37	72	0	34	30	0
Mean	54.1	67.5	0	48.8	56.6	0
Standard error	6.9	7.9	0	5.7	6.6	0
Stimulatory index	1.3			1.2		

^a Control: 1.1 mg/ml PVM.

^b Test (I): 1.1 mg/ml PVM + 0.5 μ units of PVS coat-protein at 280 nm.

^c Same concentration was used as in the *Test* inoculum.

^d Test (III): 1.1 mg/ml PVM + 1.0 μ units of PVS coat-protein at 280 nm.

TABLE 14. Simultaneous application of purified potato virus S (PVS) and potato virus M (PVM) coat-protein to *Chenopodium quinoa*.

Half-leaf no.	Lesion no. / half leaf					
	<i>Expt. I</i>			<i>Expt. II</i>		
	C ^a	T ^b	Coat-protein only ^c	C ^a	T ^d	Coat-protein only ^c
1	56	70	0	54	88	0
2	126	171	0	43	49	0
3	49	73	0	101	64	0
4	84	86	0	80	123	0
5	46	66	0	168	121	0
6	100	97	0	94	158	0
7	59	49	0	69	61	0
8	93	126	0	72	78	0
9	69	113	0	93	79	0
10	76	132	0	91	114	0
11	141	93	0	116	135	0
12	111	116	0	120	93	0
Mean	84.2	99.3	0	91.8	96.9	0
Standard error	8.9	9.9	0	9.6	9.6	0
Stimulatory index	1.2			1.1		

^a Control: 0.3 mg/ml PVS.

^b Test (I): 0.3 mg/ml PVS + 1.0 A units of PVM coat-protein at 280 nm.

^c Same concentration was used as in the Test inoculum.

^d Test (II): 0.3 mg/ml PVS + 1.5 A units of PVM coat-protein at 280 nm.

FIG. 16. Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM)-ribonucleic acid (RNA) in 'Red Kidney' bean.

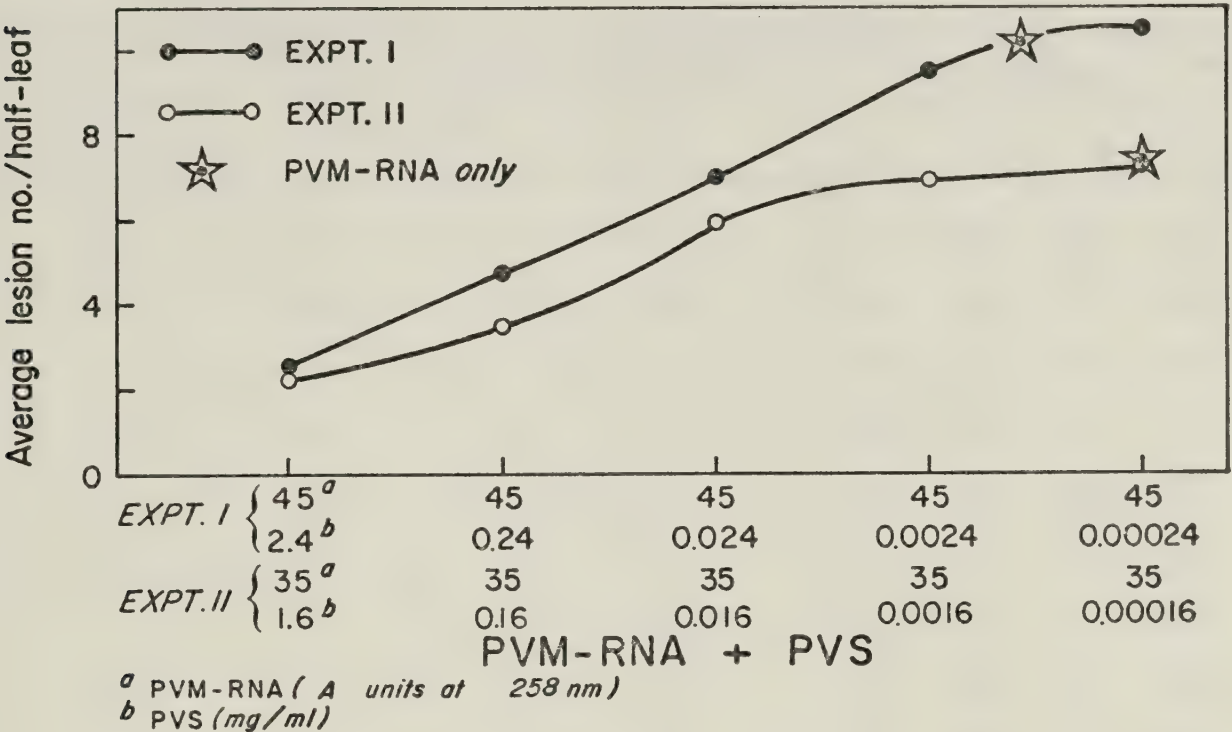


FIG. 17. Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa*.

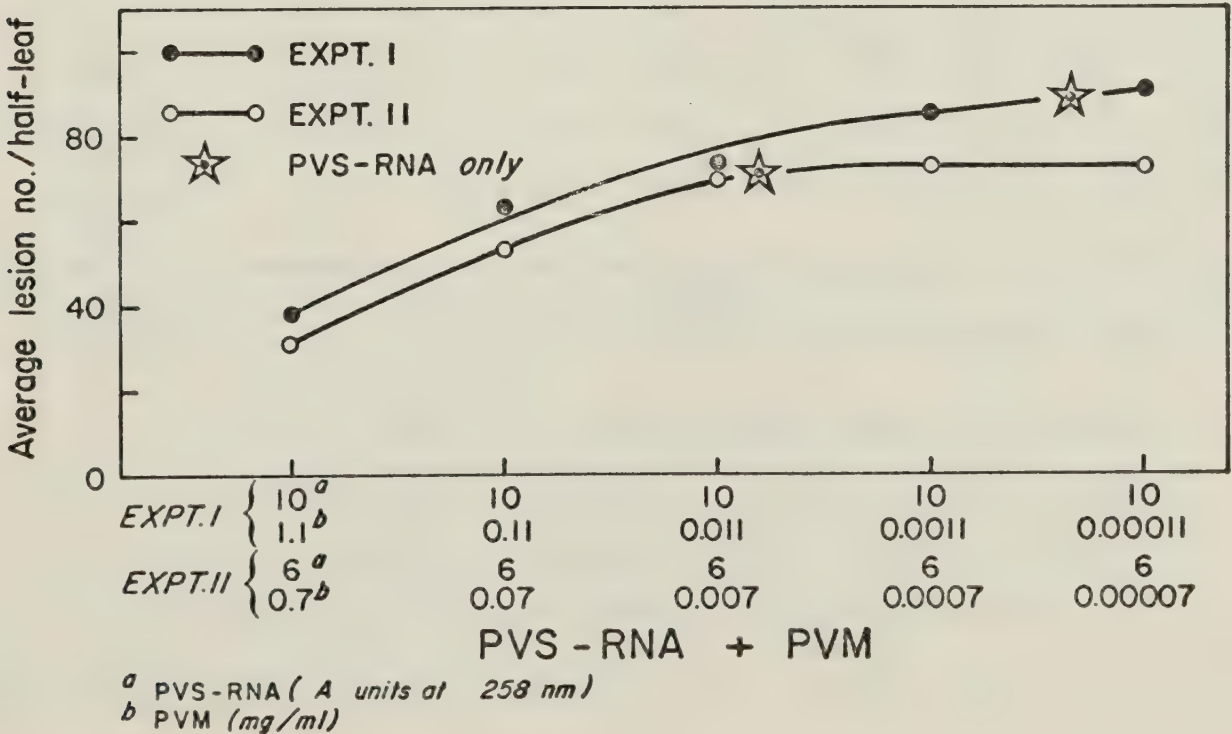


TABLE 15. The effect of endogenous ribonuclease associated with the purified potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa* in the presence of yeast-RNA.

Half-leaf no.	Lesion no./half-leaf							
	<i>Expt. I</i>				<i>Expt. II</i> ^c			
	C ^a	T ₁ ^b	T ₂	T ₃	C	T ₁	T ₂	T ₃
1	27	6	8	21	115	56	60	69
2	30	7	10	16	134	42	79	106
3	67	17	16	35	63	17	23	38
4	56	11	21	51	49	14	32	30
5	71	16	33	58	102	44	50	61
6	99	20	24	42	91	31	73	65
7	24	5	9	16	146	50	102	135
8	38	8	18	20	177	63	81	89
9	27	6	19	14	68	36	24	34
10	49	6	21	29	58	20	40	37
11	63	11	26	24	43	26	49	59
12	52	12	30	29	75	36	62	92
Mean	50.3	10.4	19.6	29.6	93.4	36.3	56.3	67.9
Standard error	6.5	1.4	2.3	4.1	12.2	4.5	7.1	9.4
Infectivity index	1.0	0.2	0.4	0.6	1.0	0.4	0.6	0.7

^a Control: 5 *A* units of PVS-RNA at 258 nm.

^b Test (1): T₁ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + phosphate buffer (0.057 M, pH 8).

T₂ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + 25 *A* units of yeast-RNA at 258 nm.

T₃ = 5 *A* units of PVS-RNA + 1.1 mg/ml PVM + 50 *A* units of yeast-RNA at 258 nm.

^c 10 *A* units of PVS-RNA were used, the rest being the same.

inhibition of the infectivity of PVS-RNA. However, the infectivity of mixed inocula never exceeded that of PVS-RNA alone.

DISCUSSION

In the mixed inoculation experiments, both PVM and PVS produced higher lesion numbers in their respective local lesion hosts in the presence of their counterparts. A plausible explanation for the increase in the lesion number might be that the counterparts, possibly their coat-proteins, played a part in activating a mechanism essential to initiate an infection, although they were unable to initiate infection by themselves in the same plants. The increase in infectivity, alternatively, may be due to a physical phenomenon. Although bioassay was the most sensitive method for testing virus, the production of a single local lesion requires about 50,000 to 1 million particles in the inoculum (Steere, 1955, 1956; Wildman, 1959). These data implied that the presence of a certain number of virus particles was essential at an infectible site to transform it to an infective centre. A hypothesis which may be worth considering is that the counterpart in the virus mixture acts in such a way that the efficiency of infection by the test virus is increased at the infectible site, thus enabling the fewer test virus particles to initiate transformation of infectible sites. This kind of stimulation would not produce a maximum effect when a test virus fully activated by the presence of the counterpart in the inoculum reaches a threshold beyond which the former becomes excessive for the infection of a host leaf where the inoculable area is

limited. This explanation seems applicable to PVS in *C. quinoa* since the increases of PVM beyond the certain level did not proportionally increase the lesion number (Fig. 12). However, in the experiment involving a host which has comparatively larger leaf area and a virus preparation of low specific infectivity, the presence of the counterpart induced a pronounced stimulatory effect which was somewhat proportional to the amount of the counterpart added to the intact virus samples (Fig. 11).

Obtaining a similar stimulatory effect, Fulton (1962) reported that *Dolichos biflorus*, which was susceptible only to the H strain and not to the G strain of sour cherry necrotic ringspot virus, produced significantly more lesions than the control when inoculated simultaneously with a mixture of the H and G strains. Even after heat- or UV-inactivation the G strain provided the same stimulatory effect. Thus, the author suggested that the stimulation of infection required more than one particle.

In the pre- and post-inoculation experiments, the counterpart stimulated the infection of the test virus to a certain extent (Figs. 13A and 13B; Table 6). Therefore, it is safe to assume from these data that some pre-inoculated virus particles remained stable until such time that it could interact with the post-inoculated virus particles. When a total number of virus particles supplemented by either a test virus or its counterpart reaches a level that is sufficient to support infection, even a few infectious test virus particles that were present in the inoculum could achieve successful infection. This stimulatory effect, however, diminished after an interval of 10 hr between applications of the test virus and its counterpart.

The results from the experiments concerning 'simultaneous' separate inoculations of upper and lower epidermal cells indicated that the separate applications of the two viruses did not induce the stimulatory effect. Thus it was clearly shown that the stimulatory effect resulted mainly from simultaneous application of the two viruses (Figs. 11 and 12) and from sequential application involving pre- and post-inoculations to some extent (Fig. 13; Table 6).

Unlike the interaction between intact viruses, simultaneous inoculation of intact virus and the viral RNA of its counterpart resulted in decreases in the local lesion number incited by the test viruses (Tables 9 and 10). Similarly, experiments with a mixture of viral RNA's yielded an inhibitory effect (Tables 11 and 12). This result suggested that genetic recombination of these two viruses was unlikely and that there was no genetic interaction between PVM and PVS even at RNA level. Therefore, the stimulation of infection using the mixed-virus-system was achieved possibly through the physical interaction of the particles between the intact viruses.

The fact that the lesion numbers obtained from the mixed-inocula of the RNA and intact viral counterpart were essentially inversely proportional to the concentration of the counterpart can be explained by the presence of RNase in the final purified virus preparations (Figs. 16 and 17). This activity of the endogenous RNase could be counteracted by adding yeast-RNA to the inoculum (Table 15). Whitfeld and Williams (1963) earlier detected a similar RNase activity present in their purified TMV preparations, and its optimal activity ranged from pH 8.5 to 9.0. In this study, a relatively high RNase activity was expected since pH 8.0 was maintained in all inocula used.

Kaper and Siberg (1969) also found the persistent presence of a trace of RNase in the purified TYMV preparations.

CHAPTER VI

CONCLUDING REMARKS

The primary objectives of investigating the methods of purification and bioassay of PVM and PVS were to obtain highly purified virus preparations and to obtain information regarding factors influencing quantitative evaluation of their infectivity so that the interaction between the two viruses can be studied accurately.

Although a certain amount of information concerning the physical properties of both viruses was obtained, the characterization of the purified viruses was by no means complete. Since relatively high virus yield can be obtained by the method used in this study, efforts can be continued along this line in the future. Studies of virus components such as protein and nucleic acid can be particularly rewarding, since both PVM and PVS have been only poorly characterized in this regard. The PVM preparations obtained in this investigation had rather low specific infectivity and the sedimentation coefficient values lower than expected. This result indicates that the PVM isolate (AP-1) used in this study is perhaps more susceptible to inactivation than the PVS isolate (A) during purification. The relatively low infectivity of the RNA isolated from PVM (Chapter V) may be due to the same reason. This situation could be remedied probably by incorporating certain chelating agents in the purification procedure, in particular

at the time of sap extraction (Hampton and Fulton, 1959, 1961; Hiruki, 1964).

The mechanism of virus precipitation by the PEG-NaCl method is unknown even though several hypotheses have been proposed. The precipitation of viruses by PEG was dependent on the difference in the charge on the surface of the virus particles (Polson et al., 1964; Albrechtová and Klír, 1970). Therefore, the PEG solubility could be controlled by adjusting the concentration or pH of the buffer solution used as a suspending medium (Clark and Lister, 1971; Reddy and Black, 1973). The PEG precipitation of proteins and viruses was comparable to the dehydrating effect of alcohol or acetone by removing the water molecules from the hydration envelope of the protein, causing a disturbance of the dielectric constant of the medium (Chun et al., 1967; Clark and Lister, 1971). As a result, the steric relationships of the hydrophobic and hydrophilic groups of the proteins were altered which led to the precipitation of the protein. Leberman (1966) proposed that virus precipitation by PEG could be due to a simple coacervation whereby a spontaneous separation of the highly hydrated polymers resulted in the formation of two phases. Iverius and Laurent (1967) hypothesized that PEG sterically excluded the virus particles from the solvent by creating a solubility limit for the virus, while Juckes (1971) suggested the PEG precipitation resembled the salting-out phenomenon.

In the present study the interaction between PVM and PVS is surprisingly different from any of the known phenomena. Thus a number of hypotheses that have been proposed (Bawden and Kassanis, 1945; Kavanau, 1949; Fulton, 1951a,b; Best 1954a,b; Damirdagh and Ross, 1967; Matthews, 1970; Wenzel, 1971; Kassanis et al., 1974) are not useful in

explaining the results. Although the counterpart viruses, alone or in association with the test viruses, are unable to establish any detectable infection in the local lesion hosts of the test viruses, they seem to participate in infection by the test viruses in an unknown manner and contribute to the increased efficiency of infection by the latter. This stimulation is highest in its effect, when the two viruses are mixed and applied simultaneously to the test plants. However, the same effect did not occur when each of them was applied separately from the upper and lower sides of the leaf tissue, or when the RNA's from these two viruses were mixed and applied to the test plants or when the intact test virus was mixed with the RNA of the counterpart and applied. The fact that the stimulatory effect of the isolated coat-proteins is considerably low when compared with that of the intact viruses may suggest the importance of protein configuration or certain requirement(s) for their molecular size. Further careful investigations, including isotopic labelling of virus and its components coupled with the immunofluorescence technique which can be effectively applicable to protoplast systems, will undoubtedly shed light to the mechanism(s) of this extremely interesting type of virus interaction.

PHOTOGRAPHIC PLATES

PLATE 1. Systemic and local lesion hosts of potato viruses M and S.

A. 50-day old systemic host, *Solanum tuberosum* L. potato plants.

M: A 'King Edward' potato plant infected with potato virus M (PVM) (Alberta isolate AP-1) showing symptoms of mild stunting.

H: A healthy 'Netted Gem' (Russet Burbank) plant in bloom.

S: A 'Netted Gem' potato plant infected with potato virus S (PVS) (Canadian isolate 'A') showing mild stunting at the blossom stage.

B. The primary leaf of *Phaseolus vulgaris* L. cv. 'Red Kidney' showing brown necrotic local lesions 8 days after inoculation with PVM. The inset shows an enlarged portion of the infected leaf.

C. A *Chenopodium quinoa* Willd. leaf 9 days after inoculation with PVS, showing local lesions with a necrotic centre and a chlorotic periphery.

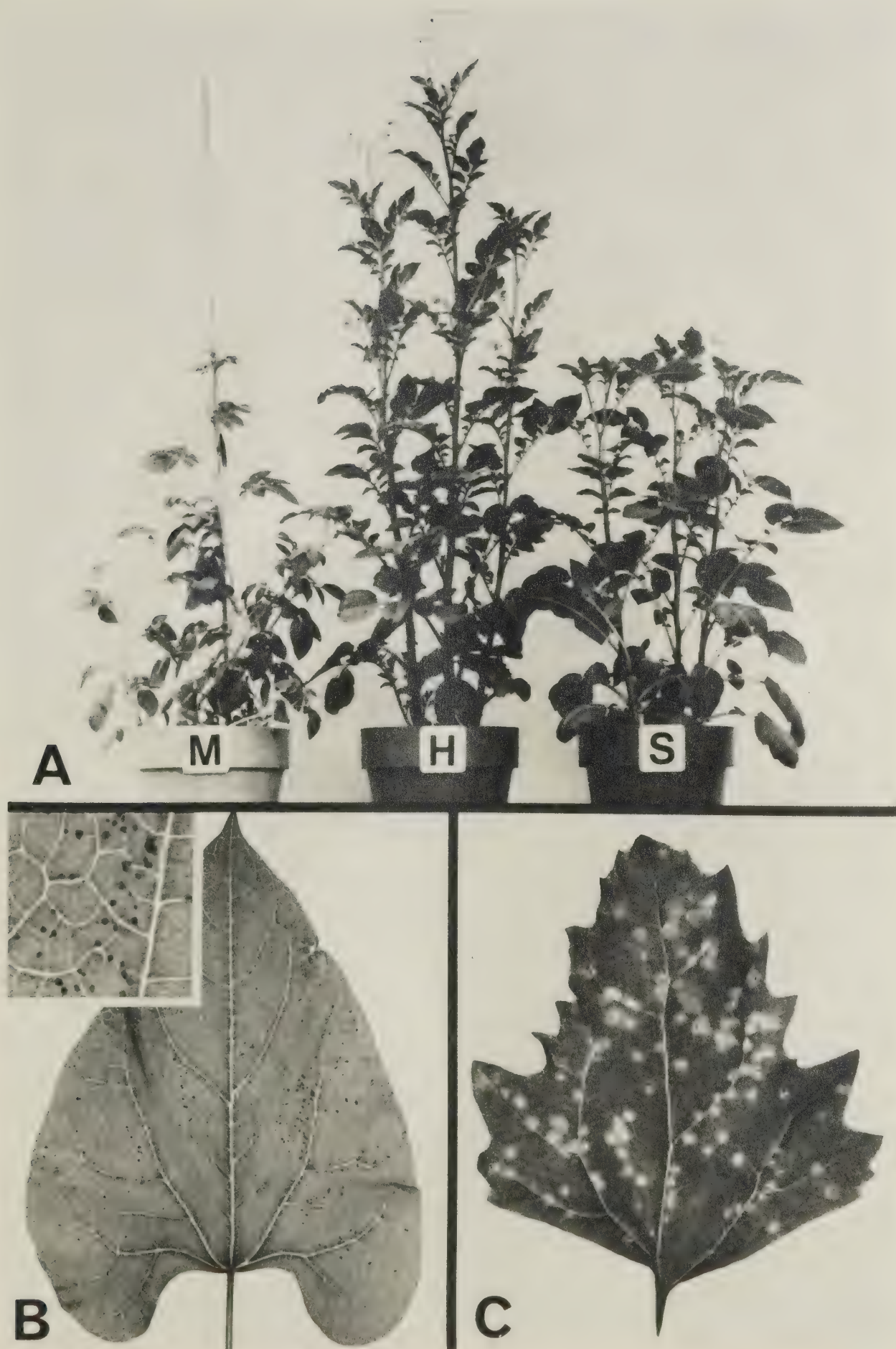


PLATE 2. Electron micrographs and length-distribution of potato virus M (PVM) obtained by the polyethylene glycol-sodium chloride purification method.

A & B. PVM particles stained with 2% sodium phosphotungstate, pH 7.0.

C. A histogram showing the length-distribution based on a measurement of 300 PVM particles.

D. Negatively stained PVM particles observed in a leaf-dip preparation.

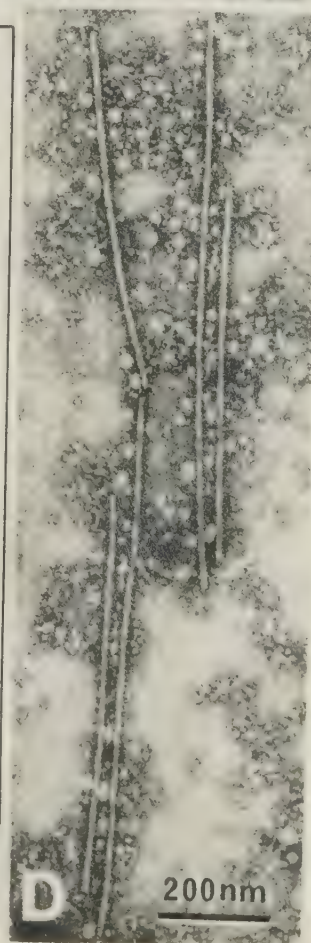
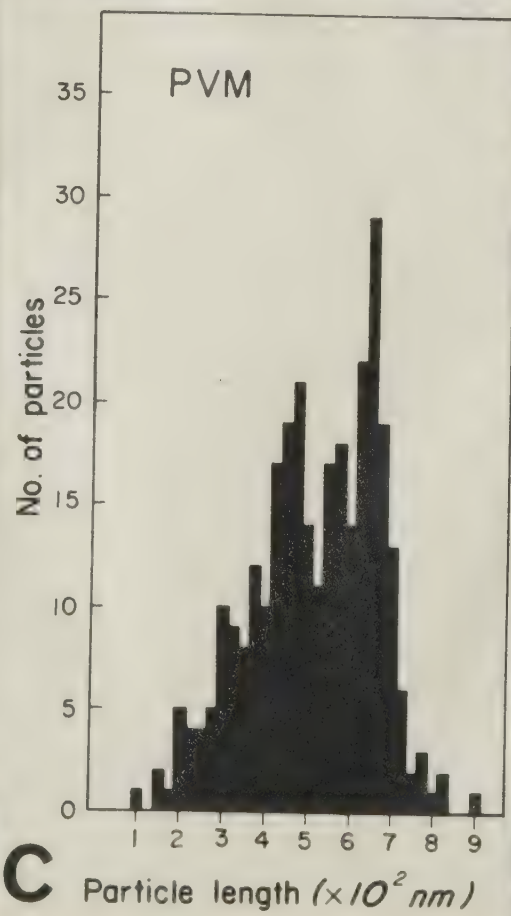
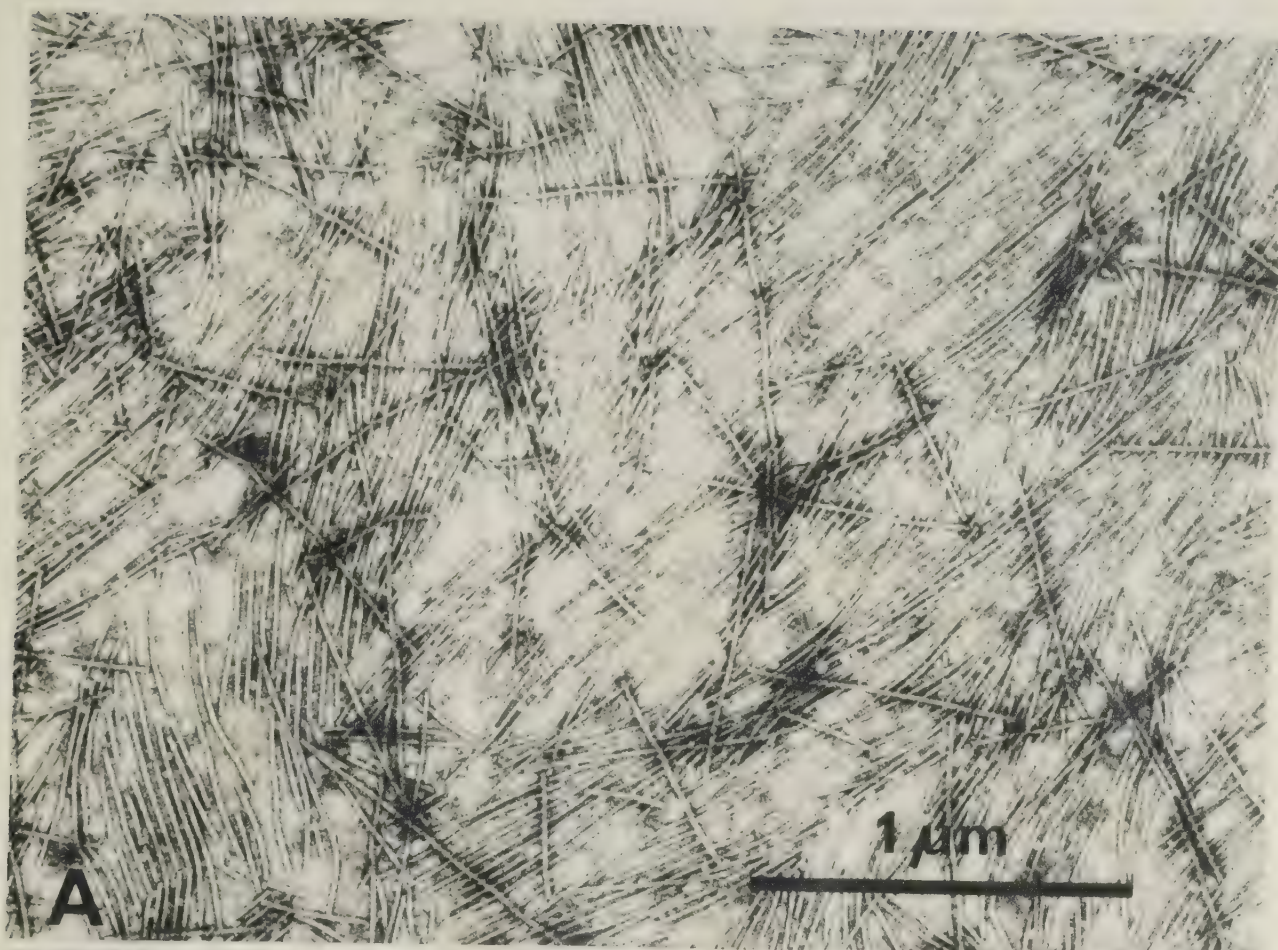
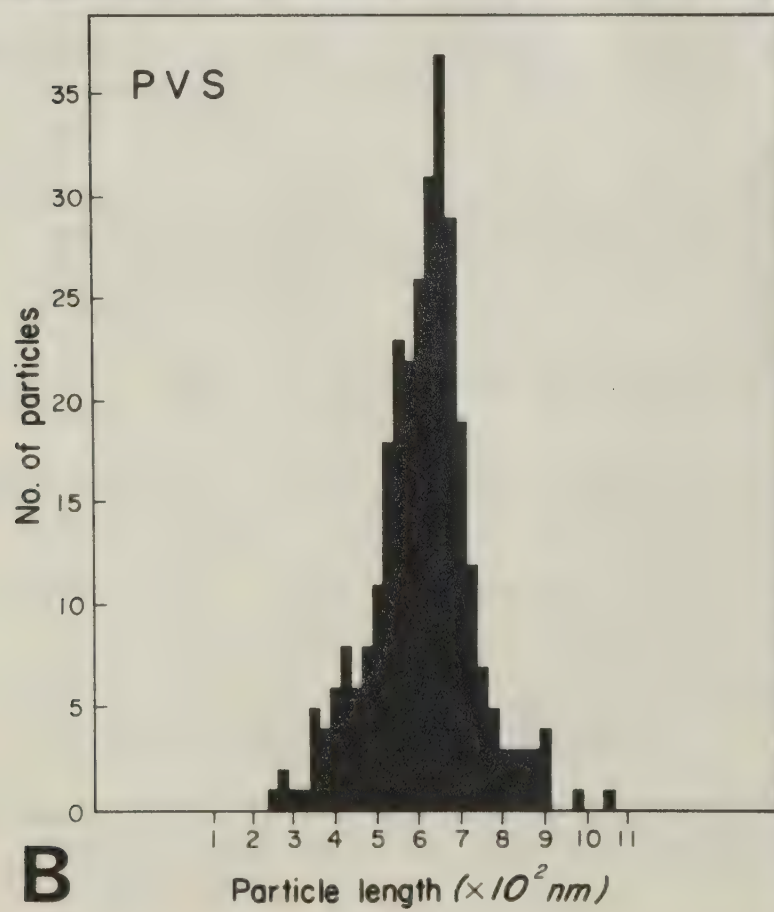
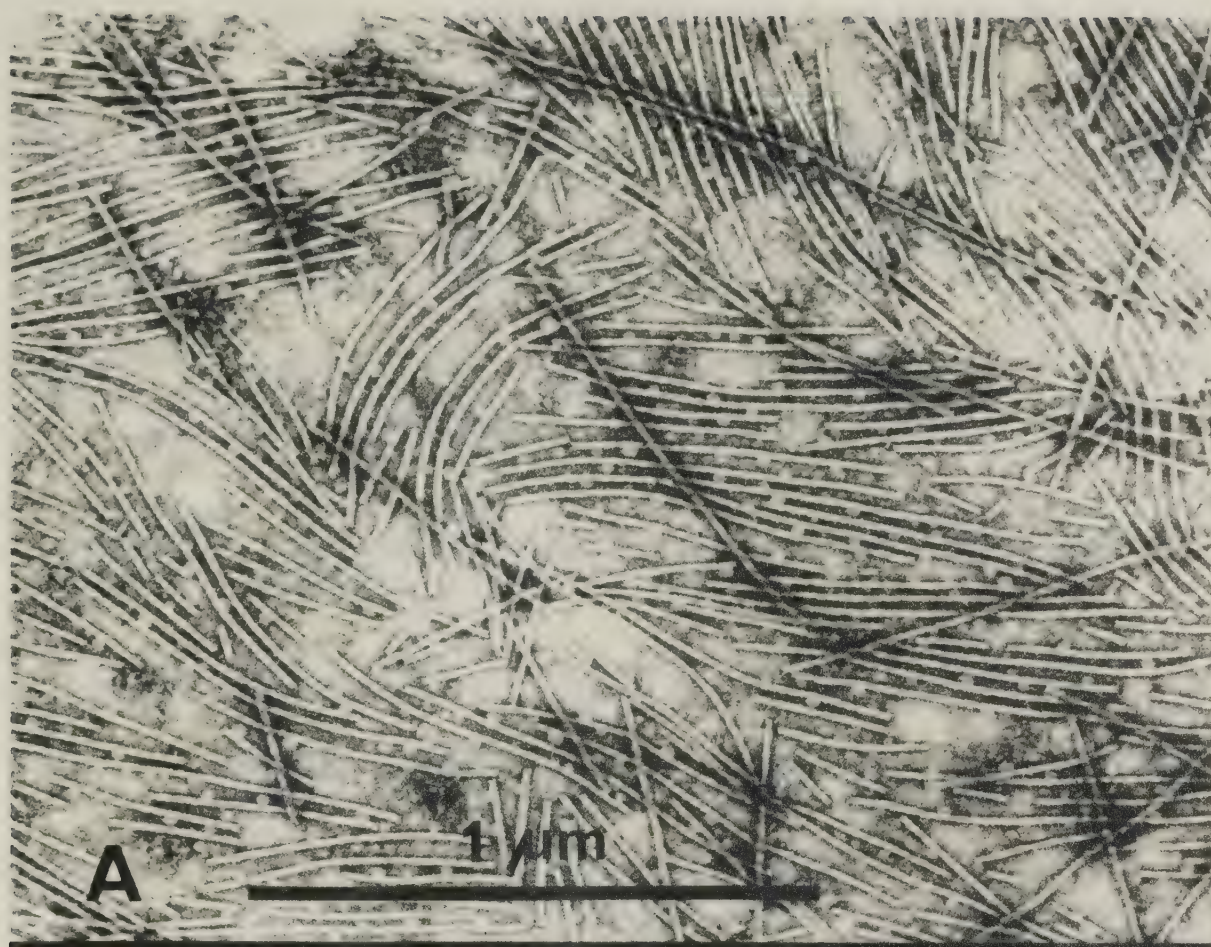


PLATE 3. Electron micrograph, length-distribution and cesium chloride (CsCl) isopycnic banding of potato virus S (PVS) obtained by the polyethylene glycol-sodium chloride purification method.

- A. PVS particles stained with 2% sodium phosphotungstate, pH 7.0.
- B. A histogram showing the length-distribution based on a measurement of 300 PVS particles.
- C. A single light scattering band obtained after density centrifugation of purified PVS preparation in 20-40% CsCl gradient.



BIBLIOGRAPHY

- ADOMAKO, D., G.K. OWUSU, and K.K. ODURO. 1974. Purification of cocoa necrosis virus from cocoa leaves. *Phytopathology* 64:1325-1330.
- ALBERTSSON, P.Å. 1960. Partition of cell particles and macromolecules. John Wiley and Sons, N.Y. 231pp.
- ALBRECHTOVÁ, L., and O. KLÍR. 1968. The isolation of potato virus S from the leaves of potato plants using precipitation by polyethylene glycol. *Biologia Plantarum* (Praha) 10:271-274.
- ALBRECHTOVÁ, L., and O. KLÍR. 1970. Precipitation of S, M, X, and Y potato viruses by polyethylene glycols with different molecular weights. *Biologia Plantarum* (Praha) 12:31-40.
- ALFIERI, S.A. 1960. Investigations on the resistance of potato virus S in *Solanum tuberosum*. (Abs.) *Diss. Abstr.* 20:4480.
- ALFIERI, S.A., and R.F. STOUFFER. 1957. Evidence of immunity from virus S in the potato variety Saco. (Abs.) *Phytopathology* 47:1.
- AMBROSOV, A.L., and O.V. SCHCHUTSKAYA. 1975. [Effect of viruses S and M on some physiological processes of potato plants.] *Cit.:Rev. Pl. Path.* 54:1020, No. 5517 (1975).
- AMBROSOV, A.L., and L.A. SOKOLOVA. 1973. [A study of the interaction between potato viruses in plant hosts.] *Cit.:Virology Abs.* 7:No. 7088 (1974).
- ANDERSON, N.G., and N.L. ANDERSON. 1970. Density at 25°C of cesium chloride solution as a function of refractive index. *In* "Handbook of Biochemistry", 2nd ed., ed. H.A. Sabar, pp. J292-296, Cleveland, Chemical Rubber Co.
- AURA, K. 1957. [Potato virus diseases in Finland.] *Cit.:Rev. appl. Mycol.* 38:220 (1959).
- BAERECHE, M.L. 1967a. [Tests of Saco-crosses for resistance to potato virus S.] *Cit.:Rev. appl. Mycol.* 47:119, No. 604 (1968).
- BAERECHE, M.L. 1967b. [Hypersensitivity to the S-virus of potato in a Bolivian andigena clone.] (Eng. sum.) *Züchter* 37:281-286.
- BAGNALL, R.H. 1965. Saco potato infected with potato virus S by grafting. *Phytopathology* 55:707.
- BAGNALL, R.H. 1972. Resistance to potato viruses M, S, X and the spindle tuber virus in tuber-bearing *Solanum* species. *Am. Potato J.* 49:342-348.

- BAGNALL, R.H., and R.H.E. BRADLEY. 1955. Note on a virus latent in potato plants. *Am. Potato J.* 32:252-253.
- BAGNALL, R.H., R.H. LARSON, and J.C. WALKER. 1956a. Three virus components of "interveinal mosaic" - Irish Cobbler potato. *Am. Potato J.* 33:271-273.
- BAGNALL, R.H., R.H. LARSON, and J.C. WALKER. 1956b. Potato viruses M, S and X in relation to interveinal mosaic of the Irish Cobbler variety. *Wisc. Agr. Exp. Sta. Res. Bull. No. 198.* 45pp.
- BAGNALL, R.H., C. WETTER, and R.H. LARSON. 1959. Differential host and serological relationships of potato virus M, potato virus S, and carnation latent virus. *Phytopathology* 49:435-442.
- BAGNALL, R.H., and D.A. YOUNG. 1960. Inheritance of immunity to virus S in potatoes. (Abs.) *Am. Potato J.* 37:311.
- BAGNALL, R.H., and D.A. YOUNG. 1968. Potato virus S-inheritance of resistance in potatoes. *Am. Potato J.* 45:188.
- BAGNALL, R.H., and D.A. YOUNG. 1972. Resistance to virus S in the potato. *Am. Potato J.* 49:196-201.
- BAILOVA-YANKULOVA, M. 1966. [Virus S on potatoes in Bulgaria.] *Cit.: Rev. appl. Mycol.* 45:395, No. 2214 (1966).
- BALD, J.G., and G. SAMUEL. 1934. Some factors affecting the inactivation rate of the virus of tomato spotted wilt. *Ann. appl. Biol.* 21:179-190.
- BANCROFT, J.B. 1962. Purification and properties of bean pod mottle virus and associated centrifugal and electrophoretic components. *Virology* 16:419-427.
- BARTELS, R. 1966. [Concentration of potato virus M in potatoes and tomatoes.] *Cit.: Rev. appl. Mycol.* 47:70, No. 395e (1968).
- BAWDEN, F.C., and B. KASSANIS. 1945. The suppression of one plant virus by another. *Ann. appl. Biol.* 32:52-57.
- BAWDEN, F.C., and B. KASSANIS. 1964. The potato variety King Edward VII and paracrinkle virus. *Rothamsted Exptl. Sta. Report (1964)*, pp. 282-290.
- BAWDEN, F.C., B. KASSANIS, and H.L. NIXON. 1950. The mechanical transmission and some properties of potato paracrinkle virus. *J. gen. Microbiol.* 4:210-219.
- BAWDEN, F.C., and N.W. PIRIE. 1950. Some effects of freezing in the leaf and of citrate *in vitro*, on the infectivity of a tobacco necrosis virus. *J. gen. Microbiol.* 4:482-492.

- BAWDEN, F.C., and N.W. PIRIE. 1957. A virus-inactivating system from tobacco leaves. *J. gen. Microbiol.* 16:696-710.
- BAWDEN, F.C., and F.M. ROBERTS. 1947. The influence of light intensity on the susceptibility of plants to certain viruses. *Ann. appl. Biol.* 34:286-296.
- BAWDEN, F.C., and F.M. ROBERTS. 1948. Photosynthesis and predisposition of plants to infection with certain viruses. *Ann. appl. Biol.* 35:418-428.
- BEEEMSTER, A.B.R., and A. ROZENDAAL. 1972. Potato viruses: properties and symptoms. *In* "Viruses of Potatoes and Seed Potato Production", ed. J.A. de Bokx, pp. 115-143. Wageningen: Centre Agric. Publ. Doc. 233 pp.
- BENNETT, C.W. 1949. Recovery of plants from dodder latent mosaic. *Phytopathology* 39:637-646.
- BENNETT, C.W. 1951. Interference phenomena between plant viruses. *Ann. Rev. Microbiol.* 5:295-308.
- BENNETT, C.W. 1953. Interactions between viruses and virus strains. *Advan. Virus Res.* 1:39-67.
- BEST, R.J. 1939. The preservation effect of some reducing systems on the virus of tomato spotted wilt. *Australian J. Exptl. Biol. Med. Sci.* 17:1-17.
- BEST, R.J. 1954a. Cross protection by strains of tomato spotted wilt virus and a new theory to explain it. *Australian J. Biol. Sci.* 7:415-424.
- BEST, R.J. 1954b. The development and multiplication of viruses. *J. Australian Inst. Agric. Sci.* 20:36-40.
- BEST, R.J. 1965. Interactions between plant viruses in their hosts: antagonism between strains of plant viruses. *Enzymologia* 29:377-389.
- BODE, O. 1948. [On a virus ("EN") isolated from tobacco with a strongly necrotic action confined to the site of infection.] *Cit.: Rev. appl. Mycol.* 28:550 (1949).
- BODE, O., and H.L. WEIDEMANN. 1971. [Further investigations on potato viruses M and S and their transmission by vectors.] (Eng. sum.) *Potato Res.* 14:119-129.
- BOS, L., D.Z. MAAT, and M. MARKOV. 1972. A biologically highly deviating strain of red clover vein mosaic virus, usually latent in pea (*Pisum sativum*), and its differentiation from pea streak virus. *Neth. J. Pl. Path.* 78:125-152.

- BRAKKE, M.K. 1951. Density gradient centrifugation: a new separation technique. J. Am. Chem. Soc. 73:1847-1848.
- BRANDES, J. 1957. [A rapid electron-microscope method for the demonstration of filamentous and rod-shaped viruses, especially in potato sprouts developed in the dark.] Cit.:Rev. appl. Mycol. 37:244 (1958).
- BRANDES, J. 1964. [Identification of elongate plant pathogenic viruses on morphological criteria with assembly of fundamental data for the classification of these viruses.] Cit.:Rev. appl. Mycol. 43:574, No. 3162 (1964).
- BRANDES, J., and C. WETTER. 1963-64. [Studies on the characteristic and relationships of passiflora latent virus] (Eng. sum.) Phytopathol. Z. 49:61-70.
- BRANDES, J., C. WETTER, R.H. BAGNALL, and R.H. LARSON. 1959. Size and shape of the particles of potato virus S, potato virus M, and carnation latent virus. Phytopathology 49:443-446.
- BRENNER, S., and R.W. HORNE. 1959. A negative staining method for high resolution electron microscopy of viruses. Biochim. Biophys. Acta 34:103-110.
- BRUNT, A.A., and R.J. BARTON. 1976. Narcissus latent virus. C.M.I./A.A.B. Descriptions of Plant Viruses. No. 170.
- BRUNT, A.A., and R.H. KENTEN. 1973. Cowpea mild mottle, a newly recognized virus infecting cowpeas (*Vigna unguiculata*) in Ghana. Ann. appl. Biol. 74:67-74.
- CHMULEV, V.M. 1974. [Photosynthesis of healthy and virus infected potato.] Cit.:Rev. Pl. Path. 54:448, No. 2404 (1975).
- CHRZANOWSKA, M. 1973. [Influence of temperature on the detectability of viruses M and S in potato plants.] (Eng. sum.) Zeszyty Problemowe Postępów Nauk Rolniczych 142:81-91.
- CHRZANOWSKA, M. 1976. Variation in symptoms caused by potato virus M on the potato variety Uran. Potato Res. 19:141-146.
- CHUN, P.W., M. FRIED, and E.F. ELLIS. 1967. Use of water-soluble polymers for the isolation and purification of human immunoglobulins. Anal. Biochem. 19:481-497.
- CLARK, M.F. 1968. Purification and fractionation of alfalfa mosaic virus with polyethylene glycol. J. gen. Virol. 3:427-432.
- CLARK, M.F. 1970. Polyethylene glycol solubility gradients, a new and rapid technique for investigations of plant viruses. Virology 42:246-247.
- CLARK, M.F., and R.M. LISTER. 1971. The application of polyethylene glycol solubility-concentration gradients in plant virus research. Virology 43:338-351.

- CLINCH, P.E.M., J.B. LOUGHNANE, and P.A. MURPHY. 1936. A study of the aucuba or yellow mosaics of the potato. *Cit.:Rev. appl. Mycol.* 16:117 (1937).
- COSTA, A.S., and C.W. BENNETT. 1955. Studies on the mechanical transmission of the yellows virus of sugar beet. *Phytopathology* 45:233-238.
- CUPERTINO, F.P., A.R. OLIVEIRA, and A.S. COSTA. 1970. [Presence of potato virus S in indigenous and foreign seed potatoes.] *Cit.:Rev. Pl. Path.* 51:329, No. 1798 (1972).
- DAMIRDAGH, I.S., and A.F. ROSS. 1967. A marked synergistic interaction of potato viruses X and Y in inoculated leaves of tobacco. *Virology* 31:296-307.
- DAMIRDAGH, I.S., and R.J. SHEPHERD. 1970. Purification of the tobacco etch and other viruses of the potato Y group. *Phytopathology* 60:132-142.
- De BOKX, J.A. 1969. Particle length of various isolates of potato virus S. *Neth. J. Pl. Path.* 75:144-146.
- De BOKX, J.A. 1970. Reactions of various plant species to inoculation with potato virus S. *Neth. J. Pl. Path.* 76:70-78.
- De BRUYN OUBOTER, M. 1952. A new potato virus. *Cit.:Rev. appl. Mycol.* 32:144 (1953).
- DICKSON, B.T. 1925. Tobacco and tomato mosaic. *Science* 62:398.
- DODDS, J.A., and R.I. HAMILTON. 1972. The influence of barley stripe mosaic virus on the replication of tobacco mosaic virus in *Hordeum vulgare* L. *Virology* 50:404-411.
- DODDS, J.A., and R.I. HAMILTON. 1976. Structural interactions between viruses as a consequence of mixed infections. *Advan. Virus Res.* 20:33-86.
- DZIEWOŃSKA, M.A., and K. OSTROWSKA. 1973. [The kidney bean var. Red Kidney as an indicator plant for detecting potato virus M. Communication.] *Cit.:Rev. Pl. Path.* 54:357, No. 1848 (1975).
- FENNER, F. 1976. The classification and nomenclature of viruses. Summary of results of meetings of the international committee on taxonomy of viruses in Madrid, Sept., 1975. *Virology* 71:371-378.
- FORD, R.E. 1967. Number and size of local lesions of alfalfa mosaic virus by interaction with bean yellow mosaic virus. *Phytopathology* 57:1274-1275.
- FRAENKEL-CONRAT, H. 1957. Degradation of tobacco mosaic virus with acetic acid. *Virology* 4:1-4.

- FRAENKEL-CONRAT, H., B. SINGER, and A. TSUGITA. 1961. Purification of viral RNA by means of bentonite. *Virology* 14:54-58.
- FREIDMANN, T., and M. HAAS. 1970. Rapid concentration and purification of polyoma virus and SV40 with polyethylene glycol. *Virology* 42:248-250.
- FROST, R.R., and B.D. HARRISON. 1967. Comparative effects of temperature on the multiplication in tobacco leaves of two tobacco rattle viruses. *J. gen. Virol.* 1:455-464.
- FRUIT, VEGETABLE AND HONEY CROP AND MARKET REPORT. 1976. 48(Dec.): 13. publ. by Market Inf. Services, Agric. Canada.
- FULTON, R.W. 1951a. The reinfection of mosaic leaves of *Nicotiana sylvestris* by other strains of tobacco mosaic virus. (Abs.) *Phytopathology* 41:13.
- FULTON, R.W. 1951b. Superinfection by strains of tobacco mosaic virus. *Phytopathology* 41:579-592.
- FULTON, R.W. 1957. Properties of certain mechanically transmitted viruses of *Prunus*. *Phytopathology* 47:683-687.
- FULTON, R.W. 1962. The effect of dilution on necrotic ringspot virus infectivity and the enhancement of infectivity by non-infective virus. *Virology* 18:477-485.
- FULTON, R.W. 1974. Recent advances in virus purification. *Proc. 3rd Int. Symp. on Virus Dis. of Ornam. Plants, and Int. Soc. of Hort. Sci. Tech. Commun.* (1972), No. 36:93-104.
- GANGULY, A. 1963. Potato virus diseases. *Cit.:Rev. appl. Mycol.* 43:75, No. 406g (1964).
- GARCES-OREJUELA, C., and G.S. POUND. 1957. The multiplication of tobacco mosaic virus in the presence of cucumber mosaic virus or tobacco ringspot virus in tobacco. *Phytopathology* 47:232-239.
- GHENA, N. 1967. [Identification of potato virus S and its distribution on various varieties in Rumania.] *Cit.:Rev. appl. Mycol.* 47:120, No. 605 (1968).
- GHENA, N. 1970. [Study of potato viruses X, Y, S and M by means of different serological reactions.] (Eng. sum.) *Ann. Phytopathol.* 2:365-377.
- GIBBS, A.J. 1969. Plant virus classification. *Advan. Virus Res.* 14:263-328.

- GIBBS, A.J., and B.D. HARRISON. 1968. Realistic approach to virus classification and nomenclature. *Nature* 218:927-929.
- GIBBS, A.J., and B.D. HARRISON. 1976. *Plant Virology, The Principles*, Publ. by Edward Arnold, London. 292 pp.
- GIBBS, A.J., B.D. HARRISON, D.H. WATSON, and P. WILDY. 1966. What's in a virus name? *Nature* 209:450-454.
- GOLD, A.H., and J.W. OSWALD. 1955. A latent virus in a potato clone resistant to virus X and its relation to potato virus S. (Abs.) *Phytopathology* 45:693-694.
- GOODING, G.V., and T.T. HEBERT. 1967. A simple technique for purification of tobacco mosaic virus in large quantities. *Phytopathology* 57:1285.
- GOODMAN, R.M., and A.F. ROSS. 1972. Enhancement by potato virus Y of potato virus X synthesis in tobacco leaves depends on the timing of systemic invasion by the viruses. (Abs.) *Phytopathology* 62:760.
- GOTH, R.W., and R.E. WEBB. 1975. Lack of potato virus S transmission via true seed in *Solanum tuberosum*. *Phytopathology* 65:1347-1349.
- HAKKAART, F.A., D.H.M. van SLOGTEREN, and N.P. de VOS.. 1962. Chrysanthemum virus B, its serological diagnosis in chrysanthemum, and its relationship to the potato viruses S and M and to carnation latent virus. *Neth. J. Pl. Path.* 63:126-135.
- HAMILTON, R.I., and J.A. DODDS. 1970. Infection of barley by tobacco mosaic virus in single and mixed infection. *Virology* 42:266-268.
- HAMPTON, R.E., and R.W. FULTON. 1959. Factors responsible for the instability of some labile plant viruses. (Abs.) *Phytopathology* 49:540.
- HAMPTON, R.E., and R.W. FULTON. 1961. The relation of polyphenol oxidase to instability *in vitro* of prune dwarf and sour cherry necrotic ringspot viruses. *Virology* 13:44-52.
- HARRISON, B.D. 1956. Studies on the effect of temperature on virus multiplication in inoculated leaves. *Ann. appl. Biol.* 44:215-226.
- HARRISON, B.D., J.T. FINCH, A.J. GIBBS, M. HOLLINGS, R.J. SHEPHERD, V. VALENTA, and C. WETTER. 1971. Sixteen groups of plant viruses. *Virology* 45:356-363.
- HEBERT, T.T. 1963. Precipitation of plant viruses by polyethylene glycol. *Phytopathology* 53:362.

- HEROLD, F. 1967. Virus investigations of potatoes from South Africa. *Am. Potato J.* 44:22-23.
- HINOSTROZA-ORIHUELA, A.M. 1973. Isolation of infectious ribonucleic acid from leaves of *Chenopodium quinoa* infected with potato virus S. *Phytopathol. Z.* 76:149-152.
- HIRUKI, C. 1964. Mechanical transmission of tobacco stunt virus. *Virology* 23:288-290.
- HIRUKI, C. 1970. Red Kidney bean, a useful bioassay host for qualitative work with potato virus M. *Phytopathology* 60:739-740.
- HIRUKI, C. 1973. Detection of potato virus M in tubers, sprouts, and leaves of potato by the French bean test. *Potato Res.* 16:202-212.
- HIRUKI, C. 1975a. Factors affecting bioassay of potato virus S in *Chenopodium quinoa*. *Phytopathology* 65:1288-1292.
- HIRUKI, C. 1975b. Host range and properties of tobacco stunt virus. *Can. J. Bot.* 53:2425-2434.
- HIRUKI, C., E. POUNTNEY, and K.N. SAKSENA. 1974. Factors affecting bioassay of potato virus M in Red Kidney bean. *Phytopathology* 64:807-811.
- HIRUKI, C., and P. SHUKLA. 1973. Intracellular location of potato virus S in leaf tissue of *Chenopodium quinoa*. *Can. J. Bot.* 51:1699-1702.
- HÖDREJÄRV, U., K. OLSPERT, and K. TARASSOVA. 1970. A method for purification of potato virus M. *Eesti NSV Tead. Akad. Toim. Biol.* 19:231-234.
- HOLLINGS, M. 1965. Disease control through virus-free stock. *Ann. Rev. Phytopathol.* 3:367-396.
- HOLMES, F.O. 1929. Inoculating methods in tobacco mosaic studies. *Bot. Gaz.* 87:56-63.
- HOLMES, F.O. 1939. "Handbook of phytopathogenic viruses", Burgess Publ. Co., Minneapolis, Minn. 221 pp.
- HOLMES, N.D., G.R. MCNAUGHTON, W.E. PHILLIPS, J.G. STOTHART, and J. WILLMAN. 1976. *Alberta Farm Guide*, pp. 136-148.
- HORIO, H., and K. KUMOI. 1975. [A mosaic disease of potato caused by potato virus S.] (Eng. sum.) *Ann. Phytopath. Soc. Japan* 41:204-214.
- HORIO, H., I. YAUO, and K. ESUMI. 1969. [Potato virus M found in Japan] (Eng. sum.) *Ann. Phytopath. Soc. Japan* 35:47-54.

- HORVÁTH, J. 1972. Symptomless *Lycopersicon* host plants for potato virus S. Am. Potato J. 49:339-341.
- HORVÁTH, J. 1973. Seed transmission experiments of potato virus M and potato virus S in *Lycopersicon* species. Acta Agron. Acad. Sci. Hung. 22:390-391.
- HOWARD, H.W., and J. WAINWRIGHT. 1960. Potato virus M and paracrinkle. Nature 186:993-994.
- HOWELL, W.E., and G.I. MINK. 1976. Host range, purification, and properties of a flexuous rod-shaped virus isolated from carrot. Phytopathology 66:949-953.
- HSU, H.T., and L.M. BLACK. 1973. Polyethylene glycol for purification of potato yellow dwarf virus. Phytopathology 63:692-696.
- HUNNIUS, W. 1972. [On the behaviour of virus M in the potato.] (Eng. sum.) Z. Pflanzenkr. Pflanzenschutz 79:385-399.
- HUTH, W., and O. BODE. 1970. [Freeing totally infected potato varieties from potato virus S infections by meristem culture.] (Eng. sum.) NachrBl. dt. PflSchutzdienst, Stuttg. 22:37-39.
- HUTTON, E.M. 1952. Some aspects of virus Y resistance in the potato (*Solanum tuberosum*). Australian J. Agr. Res. 3:362-371.
- IVERIUS, P.H., and T.C. LAURENT. 1967. Precipitation of some plasma proteins by the addition of dextran or polyethylene glycol. Biochim. Biophys. Acta 133:371-373.
- JERMOLJEV, E., and V. PRŮŠA. 1958. [Investigation of the occurrence of potato virus S in CSR and the preparation of antisera against it.] (Eng. sum.) (Abs.) Preslia 30:359-360.
- JOHNSON, J. 1927. The classification of plant viruses. Wisc. Agr. Exp. Sta. Res. Bull. No. 76.
- JUCKES, I.R.M. 1971. Fractionation of proteins and viruses with polyethylene glycol. Biochim. Biophys. Acta 229:535-546.
- KAHN, R.P., and R.L. MONROE. 1970. Virus infection in plant introductions collected as vegetative propagations. I. Wild vs. cultivated *Solanum* species. Bull. FAO Pl. Prot. 18:97-103.
- KAHN, R.P., H.A. SCOTT, J. BOZICEVICH, and M.M. VINCENT. 1967. Detection of potato viruses X, M, and S in dormant potato tubers by the bentonite flocculation test. Phytopathology 57:61-65.
- KAMERAZ, A.Y., and N.M. SHCHERBAKOVA. 1957. [Virus S and the results of its determination in potato leaves by serological method.] Cit.:Rev. appl. Mycol. 38:538 (1959).

- KANAREK, A.D., and G.W. TRIBE. 1967. Concentration of certain myxo-viruses with polyethylene glycol. *Nature* 214:927-928.
- KAPER, J.M., and R.A. SIBERG. 1969. Degradation of turnip yellow mosaic virus by freezing and thawing *in vitro*: a new method for studies on the internal organization of the viral components and for isolating native RNA. *Virology* 38:407-413.
- KASSANIS, B. 1949. The transmission of sugar beet yellows virus by mechanical inoculation. *Ann. appl. Biol.* 36:270-272.
- KASSANIS, B. 1955. Carnation latent virus. *Proc. 2nd Conf. Potato Virus Dis., Lisse-Wageningen (1954)*, pp. 134-136.
- KASSANIS, B. 1956. Serological relationship between potato paracrinkle virus, potato virus S and carnation latent virus. *J. gen. Microbiol.* 15:620-628.
- KASSANIS, B. 1957. The use tissue cultures to produce virus-free clones from infected potato varieties. *Ann. appl. Biol.* 45:422-427.
- KASSANIS, B. 1960. Potato virus M and paracrinkle. *Nature* 188:688.
- KASSANIS, B. 1961a. Potato paracrinkle virus. *Cit.:Rev. appl. Mycol.* 40:555 (1961).
- KASSANIS, B. 1961b. The transmission of potato aucuba mosaic virus by aphids from plants also infected by potato viruses A or Y. *Virology* 13:93-97.
- KASSANIS, B. 1962. Properties and behaviour of a virus depending for its multiplication on another. *J. gen. Microbiol.* 27:477-488.
- KASSANIS, B. 1963. Interactions of viruses in plants. *Advan. Virus Res.* 10:219-255.
- KASSANIS, B., S. GIANINAZZI, and R.F. WHITE. 1974. A possible explanation of the resistance of virus-infected tobacco plants to second infection. *J. gen. Virol.* 23:11-16.
- KASSANIS, B., and H.L. NIXON. 1961. Activation of one tobacco necrosis virus by another. *J. gen. Microbiol.* 25:459-471.
- KASSANIS, B., and R.F. WHITE. 1972. Interference between two satellite viruses of tobacco necrosis virus. *J. gen. Virol.* 17:177-183.
- KAVANAU, J.L. 1949. On correlation of the phenomena associated with chromosomes, foreign proteins and viruses. III. Virus-associated phenomena, characteristics and reproduction. *Am. Naturalist* 83:113-138.

- KIMMINS, W.C. 1967. The effect of darkening on the susceptibility of French bean to tobacco necrosis virus. *Can. J. Bot.* 45:543-553.
- KIMMINS, W.C., and R.E. LITZ. 1967. The effect of leaf water balance on the susceptibility of French bean to tobacco necrosis virus. *Can. J. Bot.* 45:2115-2118.
- KNESEK, J.E., G.I. MINK, and R.O. HAMPTON. 1974. Purification and properties of pea seed-borne mosaic virus. *Phytopathology* 64:1076-1081.
- KÖHLER, E. 1935. [Experimental observations on the degeneration under field conditions of potatoes (Kl.-Sp. Wohltmann variety) artificially infected by leaf roll. (Investigations on the virus diseases of the potato. Note V.)] *Cit.:Rev. appl. Mycol.* 15:246 (1936).
- KÖHLER, E. 1941. [An overlooked potato virus.] *Cit.:Rev. appl. Mycol.* 20:486 (1941).
- KÖHLER, E. 1953. [An unknown potato virus.] *Cit.:Rev. appl. Mycol.* 33:620 (1954).
- KÖHLER, E. 1955. [An unknown potato virus.] *Cit.:Rev. appl. Mycol.* 34:807 (1955).
- KÖHLER, E. 1969. [Notes on the problem of virus interference in plants.] (Eng. sum.) *Phytopathol. Z.* 64:175-197.
- KOJIMA, M., and T. TAMADA. 1976. Purification and serology of soy-bean dwarf virus. *Phytopathol. Z.* 85:237-250.
- KOLOBAEV, V.A. 1963. [Potato disease caused by leaf rolling and features of its causal agent.] *Cit.:Rev. appl. Mycol.* 44:223, No. 1203 (1965).
- KOWALSKA, A., and S. SKRZECZKOWSKA. 1976. The effect of some factors on the efficiency of potato virus S detection on detached leaves of *Solanum demissum* Y. *Phytopathol. Z.* 85:280-286.
- KOWALSKA, A., and M. WAŚ. 1976. Detection of potato virus M and potato virus S on test plants. *Potato Res.* 19:131-139.
- KOZAR, F.E., and N.A. SIVERS. 1975. [Utilization of Triton X-100 for purification of potato virus S.] (Eng. sum.) *Mikrobiol. Zhurnal.* 37:240-242.
- KRISTENSEN, H.R. 1956. [Potato virus S] *Cit.:Rev. appl. Mycol.* 35:708 (1956).

- LA, Y.J. 1974. [Serological identification of potato viruses in Korea.] Cit.:Rev. Pl. Path. 54:936, No. 5045 (1975).
- LARSON, R.H., and N. OSHIMA. 1959. Potato virus S recovered from the roots of the immune variety 'Saco'. (Abs.) Am. Potato J. 36:299.
- LASTRA, R., and K. MUNZ. 1969. Purification and electron microscopy of squash mosaic virus. Phytopathology 59:1429-1435.
- LEBERMAN, R. 1966. The isolation of plant viruses by means of "simple" coacervates. Virology 30:341-347.
- LEE, S.T. 1971. Occurrence of potato virus M and its strains in Taiwan. Cit.:Rev. Pl. Path. 52:401, No. 2002 (1973).
- LETAL, J.R., and C. HIRUKI. 1976. Alberta seed potato improvement program. Agrc. Bull., Univ. Alberta, Canada. 1976(Fall):2-7.
- LIM, S.M. 1967. The effect of latent virus diseases on the growth, yield, and quality of Sebago potatoes. Diss. Abstr. 278:2567.
- LIM, S.M., N.R. THOMPSON, and W.J. HOOKER. 1966. The effect of latent virus mosaic diseases on the growth, yield and quality of Sebago potatoes. Am. Potato J. 43:344.
- LIMASSET, P., and P. CORNUET. 1949. [Search for the virus of tobacco mosaic (*Marmor tabaci* Holmes) in the meristems of infected plants.] Cit.:Rev. appl. Mycol. 28:549 (1949).
- LOEBENSTEIN, G. 1972a. Localization and induced resistance in virus-infected plants. Ann. Rev. Pl. Pathol. 10:177-206.
- LOEBENSTEIN, G. 1972b. Inhibition, interference and acquired resistance during infection. In "Principles and Techniques in Plant Virology", ed. C.I. Kado and H.O. Agrawal, pp. 32-61, Van Nostrand Reinhold Co., N.Y.
- LORING, H.S. 1937. Accuracy in the measurement of the activity of tobacco mosaic virus protein. J. Biol. Chem. 121:637-647.
- LOUGHNANE, J.B. 1958. A necrosis of potato tubers caused by the paracrinkle virus. Proc. 3rd Conf. Potato Virus Dis., Lisse-Wageningen (1957), pp. 179-183.
- LOVISOLO, O., and M.P. BENETTI. 1959. [On the presence in Italy of potato viruses S and M.] Cit.:Rev. appl. Mycol. 40:378 (1961).
- LWOFF, A., and P. TOURNIER. 1971. Remarks on the classification of viruses. In "Comparative Virology", ed. K. Maramorosch and E. Kurstak. pp. 1-42, Academic Press, N.Y.

- MACARTHUR, A.W. 1956. Potato virus S. *Cit.:Rev. appl. Mycol.* 35:919 (1956).
- MACDONALD, D.M. 1973. Heat treatment and meristem culture as a means of freeing potato varieties from viruses X and S. *Potato Res.* 16:263-269.
- MACKINNON, J.P. 1974. Detection, spread, and aphid transmission of potato virus S. *Can. J. Bot.* 52:461-465.
- MACKINNON, J.P., and R.H. BAGNALL. 1972. Use of *Nicotiana debneyi* to detect viruses S, X and Y in potato seed stocks and relative susceptibility of six common varieties to potato virus S.
- MACKINNON, J.P., J.E. CAMPBELL, and R.E. Longmoore. 1972. Multiplication and rate of reinfection of virus-free potatoes in Prince Edward Island. *Am. Potato J.* 49:432-437.
- MACLEOD, D.J. 1939. Viruses isolated from potato varieties in cultivation in Canada. *Can. Pl. Disease Survey* 19:69-74.
- MACLEOD, D.J. 1962. Mosaic and streak viruses of the potato. *Publ. Dept. Agr. Can. No. 1150.* 80 pp.
- MAKAROV, P.P. 1975. Inheritance of resistance to potato virus S. *Potato Res.* 18:326-329.
- MARKHAM, R. 1960. A graphical method for the rapid determination of sedimentation coefficients. *Biochem. J.* 77:516-519.
- MARROU, J., and A. MIGLIORI, A. 1971 [Interaction between cucumber mosaic and tobacco mosaic viruses. III.-CMV and TMV content in tobacco leaves when the two viruses are inoculated at the same time.] (Eng. sum.) *Ann. Phytopathol.* 3:439-445.
- MATKIN, O.A., and P.A. CHANDLER. 1957. The U.C.-type soil mixtures. *In* "The U.C. System for Producing Healthy Container-Grown Plants", ed. K.F. Baker, pp. 68-85, Calif. Agric. Exp. Sta. Serv. Man. No. 23.
- MATTHEWS, R.E.F. 1970. *Plant Virology.* Academic Press, N.Y., 778 pp.
- MCCLELLAND, C.P., and R.L. BATEMAN. 1945. Technology of the polyethylene glycols and Carbowax compounds. *Chem. and Engg. News.* 23:247-251.
- MCCLENDON, J.H. 1954. Physical environment of chloroplasts as related to their morphology and activity *in vitro*. *Plant Physiol.* 24:448-458.
- MCKAY, M.B., and T.P. DYKSTRA. 1932. Potato virus diseases—Oregon investigations 1924-1929. *Oregon Agr. Exp. Sta. Bull.* 294:1-40.

- MCKEE, R.K. 1964. Virus infection in South American potatoes. Cit.:Rev. appl. Mycol. 44:101, No. 526 (1965).
- MCKINNEY, H.H. 1941. Virus-antagonism tests and their limitations for establishing relationships between mutants, and nonrelationships between distinct viruses. Am. J. Bot. 28:770-778.
- MCKINNEY, H.H., and G. SILBER. 1968. Methods in preservation and storage of plant viruses. In "Methods in Virology", ed. K. Maramorosch and H. Koprowski, vol. 4, pp. 491-501, Acad. Press, N.Y.
- MCSHARRY, J., and R. BENZINGER. 1970. Concentration and purification of vesicular stomatitis virus by polyethylene glycol "precipitation". Virology 40:745-746.
- MCWHORTER, F.P., and W.C. PRICE. 1949. Evidence that two different plant viruses can multiply simultaneously in the same cell. Science 109:116-117.
- MELLOR, F.C., and R. STACE-SMITH. 1967. Eradication of potato virus X by thermotherapy. Phytopathology 57:674-678.
- MELLOR, F.C., and R. STACE-SMITH. 1970. Virus strain differences in eradication of potato viruses X and S. Phytopathology 60:1587-1590.
- MELLOR, F.C., and R. STACE-SMITH. 1971. Heat-stable strains of potato leaf roll virus. Phytopathology 61:246-247.
- MENDOZA, H.A. 1967. [Potato virus S in Peru.] Cit.:Rev. appl. Mycol. 48:666, No. 3620 (1969).
- MERYMAN, H.T. 1956. Mechanics of freezing in living cells and tissues. Science 124:515-521.
- MESELSON, M., F.W. STAHL, and J. VINOGRAD. 1957. Equilibrium sedimentation of macromolecules in density gradients. Proc. Natl. Acad. Sci., U.S. 43:581-588.
- MOREL, G. 1948. [Investigations on the associate culture of obligate parasites and plant tissues.] Cit.:Biol. Abs. 24:No.31019 (1950).
- MOREL, G. 1964. [Regeneration of virus-infected varieties by culture of apical meristems.] Cit.:Rev. appl. Mycol. 44:193, No. 1020 (1965).
- MOREL, G., and J.F. MULLER. 1964. La culture *in vitro* du méristème apical de la pomme de terre. C.R. Acad. Sci. Paris 258:5250-5252.

- MUNRO, J. 1954. Maintenance of virus X-free potatoes. *Am. Potato. J.* 31:73-82.
- MURASHIGE, T., and F. SKOOG. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum* 15:473-497.
- NITZANY, F.E., and S. COHEN. 1960. A case of interference between alfalfa mosaic virus and cucumber mosaic virus. *Virology* 11:771-773.
- NITZANY, F.E., and I. SELA. 1962. Interference between cucumber mosaic virus and tobacco mosaic virus on different hosts. *Virology* 17:549-553.
- OSHIMA, N., and R. SATO. 1965. [Size and shape of the particles of potato viruses X, Y, F, and S.] (Eng. sum.) *Hokkaido Nat. Agr. Exp. Res. Bull. No. 86*, pp. 57-63.
- OSWALD, J.W., A. ROZENDAAL, and J.P.H. van der WANT. 1955. The alfalfa mosaic virus in the Netherlands, its effect on potato and a comparison with the potato aucuba mosaic virus. *Proc. 2nd Conf. Potato Virus Dis. Lisse-Wageningen (1954)*, pp. 137-147.
- OTSUKI, Y., and I. TAKEBE. 1973. [Multiple infection of tobacco protoplasts by TMV, CMV and PVX.] (Abs.) (in Japanese). *Ann. Phytopath. Soc. Japan* 39:244.
- OTSUKI, Y., and I. TAKEBE. 1974. [Double infection of tobacco protoplasts by ordinary and tomato strains of TMV.] (Abs.) (in Japanese) *Ann. Phytopath. Soc. Japan* 40:210-211.
- OTSUKI, Y., and I. TAKEBE. 1976. Double infection of isolated tobacco mesophyll protoplasts by unrelated plant viruses. *J. gen. Virol.* 30:309-316.
- PALIWAL, Y.C., and J.H. TREMAINE. 1976. Multiplication, purification and properties of ryegrass mosaic virus. *Phytopathology* 66:406-414.
- PAYEN, O., and P. MADEC. 1957. [On the presence in France of a new virus of the potato.] *Cit.:Rev. appl. Mycol.* 37:110 (1958).
- PETT, B. 1974. [Investigations on meristem culture of potatoes.] *Cit.:Rev. Pl. Path.* 54:273, No. 1397 (1975).
- PHILIPSON, L., P.Å. ALBERTSSON, and G. FRICK. 1960. The purification and concentration of viruses by aqueous polymer phase systems. *Virology* 11:553-571.
- POLSON, A., G.M. POTGIETER, J.F. LARGIER, G.E.F. MEARS, and F.I. JOUBERT. 1964. The fractionation of protein mixtures by linear polymers of high molecular weight. *Biochim. Biophys. Acta* 82: 463-475.

- PONTIS, R.E., and J.M. FELDMAN. 1963. [Potato virus M in Mendoza Province, Argentina.] *Cit.:Rev. appl. Mycol.* 44:153, No. 796 (1965).
- PRICE, W.C. 1935. Acquired immunity from cucumber mosaic in zinnia. *Phytopathology* 25:776-789.
- PRICE, W.C. 1940. Acquired immunity from plant virus diseases. *Quart. Rev. Biol.* 15:338-361.
- PRICE, W.C. 1964. Strains, mutation, acquired immunity, and interference. *In* "Plant Virology", ed. M.K. Corbett and H.D. Sisler, pp. 93-117, Univ. Florida Press. Gainesville.
- Priode, C.N. 1928. Further studies in the ring-spot disease of tobacco. *Am. J. Bot.* 16:88-93.
- QUAK, F. 1961. Heat treatment and substances inhibiting virus multiplication, in meristem culture to obtain virus-free plants. *Advan. Hort. Sci. Appl.* 1: 144-148.
- QUAK, F. 1972. Therapy. *In* "Viruses of Potatoes and Seed Potato Production", ed. J.A. de BOKX, pp. 158-166. Wageningen; Centre Agr. Publ. Doc. 233 pp.
- RAO, D.V. 1977. A vetch isolate of clover yellow mosaic virus: identification, infectivity assay and multiplication. Ph. D. Thesis. Univ. Alberta, Canada.
- REDDY, D.V.R., and L.M. BLACK. 1973. Estimate of absolute specific infectivity of wound tumor virus purified with polyethylene glycol. *Virology* 54:150-159.
- RIEMAN, G.H. 1956. Early history of potato seed certification in North America, 1913-1922. *Potato Handbook*, New Brunswick: Potato Assoc. Am. 1:6-10.
- ROCHOW, W.F. 1954. Multiplication of unrelated plant viruses in double infections. *Diss. Abstr.* 14:1890-1891.
- ROCHOW, W.F., and A.F. ROSS. 1954. Relative concentration of potato virus X in double and single infections. (Abs.) *Phytopathology* 44:504.
- ROCHOW, W.F., and A.F. ROSS. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology* 1:10-27.
- ROSS, A.F. 1950. Local lesion formation and virus production following simultaneous inoculation with potato viruses X and Y. (Abs.) *Phytopathology* 40:24.

- ROSS, A.F. 1953. *Physalis floridana* as a local lesion test plant for potato virus Y. *Phytopathology* 43:1-8.
- ROSS, A.F. 1957. Responses of plants to concurrent infection by two or more viruses. *Trans. N.Y. Acad. Sci.* 19:236-243.
- ROSS, A.F. 1959. The interaction of viruses in the host. In "Plant Pathology; Problems and Progress, 1908-1958", ed. C.S. Holton, G.W. Fisher, R.W. Fulton, H. Hart and S.E.A. McCallan, pp. 511-520, Univ. Wisconsin Press, Madison.
- ROSS, A.F. 1961. Localized acquired resistance to plant virus infection in hypersensitive hosts. *Virology* 14:329-339.
- ROSS, A.F. 1974. Interactions of viruses in the host. *Proc. 3rd Int. Symp. on Virus Dis. of Ornam. Pl., and Int. Soc. of Hort. Sci. Tech. Comm.* (1972) 36:247-260.
- ROSS, H. 1968. [*Lycopersicon chilense*, a test plant for the two potato viruses M and S.] *Cit.:Rev. appl. Mycol.* 48:224, No. 1273 (1969).
- ROZENDAAL, A. 1954. [The significance of different virus groups in seed potato production.] *Cit.:Rev. appl. Mycol.* 34:474 (1955).
- ROZENDAAL, A., and J.H. BRUST. 1955. The significance of potato virus S in seed potato culture. *Proc. 2nd Conf. Potato Virus Dis., Lisse-Wageningen* (1954), pp. 120-133.
- ROZENDAAL, A., and D.H.M. van SLOGTEREN. 1958. A potato virus identified with potato virus M and its relationship with potato virus S. *Proc. 3rd. Conf. Potato Virus Dis., Lisse-Wageningen* (1957), pp. 20-36.
- SADASIVAN, T.S. 1940. A quantitative study of the interaction of viruses in plants. *Ann. appl. Biol.* 27:359-367.
- SALAMAN, R.N., and R.H. Le PELLEY. 1930. Paracrinkle: a potato disease of the virus group. *Proc. Roy. Soc. Lond. Ser. B*, 106: 140-175.
- SAMPSON, P.J., and R.H. TAYLOR. 1968. A comparison of the electron microscope, microprecipitin tests, and indicator plants for the detection of potato viruses S, X, and Y. *Phytopathology* 58: 489-493.
- SAMUEL, G., and J.G. BALD. 1933. On the use of primary lesions in quantitative work with two plant viruses. *Ann. appl. Biol.* 20: 70-99.
- SCHNEIDER, I.R. 1953. Solution of tobacco mosaic virus in the aqueous phase of a chloroform-water emulsion and application of this phenomenon in virus assay. *Science* 117:30-31.

- SCHOLZ, M. 1962. [The significance of virus S for potato growing and the problem of virus S sanitation.] *Cit.:Rev. appl. Mycol.* 42:213 (1963).
- SCHOLZ, M. 1964. [The resistance behaviour of some potato varieties to virus S and the possibility of keeping potato breeding stocks free from it.] *Cit.:Rev. appl. Mycol.* 44:306, No. 1648 (1965).
- SCHOLZ, M. 1965a. [Differentiation of potato viruses S and M, and the occurrence of virus M in potato varieties of the DDR.] *Cit.:Rev. appl. Mycol.* 45:97, No. 560 (1966).
- SCHOLZ, M. 1965b. [Factors which influence the serological diagnosis of potato virus S in field and glasshouse plants.] *Cit.:Rev. appl. Mycol.* 44:573, No. 3131 (1965).
- SCHULTZ, E.S. 1951. Interveinal mosaic of potato. (Abs.) *Phytopathology* 41:564-565.
- SCHULTZ, E.S., and D. FOLSOM. 1923. Transmissions variation, and control of certain degeneration diseases of Irish potatoes. *J. Agr. Res.* 25:43-117.
- SEED POTATOES FROM CANADA. 1975. CDA, Central Exp. Farm, Ottawa, Dept. of Industry, Trade and Commerce.
- SHEPARD, J.F. 1970. A radial immunodiffusion test for the simultaneous diagnosis of potato viruses S and X. *Phytopathology* 60:1669-1671.
- SHEPARD, J.F., and L.E. CLAFLIN. 1975. Critical analyses of the principles of seed potato certification. *Ann. Rev. Phytopath.* 13:271-293.
- ŠÍP, V. 1974. [The spread of potato virus S under field conditions] *Cit.:Rev. Pl. Path.* 54:448, No. 2406 (1975).
- ŠÍP, V., and M. HAVLOVÁ. 1969. [Damage due to potato virus S.] *Cit.:Rev. Pl. Path.* 49:148, No. 825 (1970).
- SMITH, K.M. 1933. The present status of plant virus research. *Biol. Rev.* 8:137-179.
- SMITH, K.M. 1937. A textbook of plant virus diseases. 1st. ed. J. & A Churchill Ltd., London. 615 pp.
- SMITH, K.M. 1945. Transmission by insects of a plant virus complex. *Nature* 155:174-175.
- SMITH, K.M. 1946. The transmission of a plant virus complex by aphids. *Parasitology* 37:131-134.
- SPIRE, D., Y. MAURY, and J. BERTRANDY. 1967. [Note on the migration

rate of potato virus S from the potato leaves to the tubers.]
(Eng. sum.) Ann. Epiphyties 18:189-191.

STACE-SMITH, R., and F.C. MELLOR. 1967. Thermostability of potato viruses X and S. (Abs.) Phytopathology 57:1009.

STACE-SMITH, R., and F.C. MELLOR. 1968a. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58:199-203.

STACE-SMITH, R., and F.C. MELLOR. 1968b. Virus-free potato: a reality. J. Can. Dept. Agr. 13:2-6.

STANLEY, W.M. 1940. Purification of tomato bushy stunt virus by differential centrifugation. J. Biol. Chem. 135:437-454.

STEERE, R.L. 1955. Concepts and problems concerning the assay of plant viruses. Phytopathology 45:196-208.

STEERE, R.L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.

STEERE, R.L. 1959. The purification of plant viruses. Advan. Virus Res. 6:1-73.

TAKEBE, I., Y. OTSUKI, Y. HONDA, and C. MATSUI. 1975. Penetration of plant viruses into isolated tobacco leaf protoplasts. In "Proceedings of the First Inter-sectional Congress of IAMS", ed. T. Hasegawa, vol. 3, pp. 55-64, Tokyo: Science Council of Japan.

TAPIO, E. 1972. Virus-free clones of the potato varieties Pito and Tammiston aikainen. Ann. Agr. Fenn., Ser. Phytopathol. 11:115-118.

THOMSON, A.D. 1958a. The elimination of viruses from potato tissue. Proc. 3rd Conf. Potato Virus Dis., Lisse-Wageningen (1957), pp. 156-159.

THOMSON, A.D. 1958b. Interference between plant viruses. Nature 181:1547-1548.

THOMSON, A.D. 1959. Potato viruses A and S in New Zealand. N.Z. J. Agr. Res. 2:702-706.

THOMSON, A.D. 1961. Effect of tobacco mosaic virus and potato virus Y on infection by potato virus X. Virology 13:262-264.

TU, J.C., and C. HIRUKI. 1970. Ultrastructure of potato infected with potato virus M. Virology 42:238-242.

UPRETI, G.C., B.B. NAGAICH, and S.B. LAL. 1969. Occurrence of potato virus S in India. Cit.:Rev. Pl. Path. 50:47, No.234 (1971).

- Van der ZAAG, D.E. 1972. Dutch techniques of growing seed potatoes. In "Viruses of Potatoes and Seed Potato Production", ed. J.A. de Bokx, pp. 188-205, Wageningen; Centre Agr. Publ. Doc. 233 pp.
- Van der ZAAG, D.E. 1976. Potato production and utilization in the world. *Potato Res.* 19:37-72.
- Van KAMMEN, A. 1967. Purification and properties of the components of cowpea mosaic virus. *Virology* 31:633-642.
- Van SLOGTEREN, D.H.M. 1955. Preparation of antisera against potato virus S with special reference to the preparation of non-toxic virus-suspensions for the immunization of rabbits from extracts of infected potato plants. *Proc. 2nd Conf. Potato Virus Dis., Lisse-Wageningen (1954)*, pp. 35-39.
- Van SLOGTEREN, D.H.M., N.P. de VOS, and F.A. HAKKAART. 1962. Serological relationship of chrysanthemum virus B to carnation latent virus and potato viruses M and S. *Nature* 195:835-836.
- Van SLOGTEREN, E. 1955. Serological diagnosis of plant virus diseases. *Ann. appl. Biol.* 42:122-128.
- VANTERPOOL, T.C. 1926. Streak or winter blight of tomato in Quebec. *Phytopathology* 16:311-331.
- VARMA, A., A.J. GIBBS, and R.D. WOODS. 1970. A comparative study of red clover vein mosaic virus and some other plant viruses. *J. gen. Virol.* 8:21-32.
- VAUGHAN, E.K., and D.H.M. van SLOGTEREN. 1956. Potato virus S in Oregon. *Am. Potato J.* 33:218-219.
- VENEKAMP, J.H., and W.H.M. MOSCH. 1963. Chromatographic studies on plant viruses. I. The isolation of potato virus X by means of various systems of adsorption chromatography. *Virology* 19:316-321.
- VENEKAMP, J.H. and W.H.M. MOSCH. 1964a. Chromatographic purification of plant viruses on cellulose columns with polyethylene glycol containing solutions as solvents. *Neth. J. Pl. Path.* 70:85-89.
- VENEKAMP, J.H., and W.H.M. MOSCH. 1964b. Chromatographic studies on plant viruses. II. The use of polyethylene glycol in clarification and purification of tobacco mosaic virus. *Virology* 22:503-507.
- VENEKAMP, J.H., and W.H.M. MOSCH. 1964c. Chromatographic studies on plant viruses. III. The purification of potato virus X, potato virus Y, tobacco mosaic virus and potato stem mottle virus by chromatography on cellulose columns with polyethylene glycol—containing solutions as solvents. *Virology* 23:394-402.

- VULIČ, M., and W. HUNNIUS. 1967a. [The 'immunity' of variety Saco towards potato virus S.] Cit."Rev. appl. Mycol. 47:119, No. 603 (1968).
- VULIČ, M., and W. HUNNIUS. 1967b. [The reactions of various plant species to leaf infection with potato viruses S and M.] (Eng. sum.) Phytopathol. Z. 59:225-248.
- WAGNER, G.G., J.L. CARD, and K.M. COWAN. 1970. Immunochemical studies of foot-and-mouth disease. VII. Characterization of foot-and-mouth disease virus concentrated by polyethylene glycol precipitation. Arch. Ges. Virusforsch. 30:343-352.
- WATSON, M.A. 1952. Beet yellows virus and other yellowing virus diseases of sugar beet. Rotham. Agr. Exp. Sta. Ann. Rept. (1951), pp. 1-11.
- WENZEL, G. 1971. [Comparative analysis of interference in phytopathogenic viruses of tobacco.] (Eng. sum.) Phytopathol. Z. 71:147-162.
- WETTER, C. 1957. [Serological studies on the distribution and concentration of S-virus potato plants.] Cit.:Rev. appl. Mycol. 36:780 (1957).
- WETTER, C. 1960. [Partial purification of some elongated plant viruses and their use as antigens in immunization by means of Freund's adjuvant.] (Eng. sum.) Archiv. für Mikrobiol. 37:278-292.
- WETTER, C. 1967. [The influence of the concentration of agar and electrolyte on the immunodiffusion of tobacco mosaic and carnation latent viruses.] Cit.:Rev. appl. Mycol. 47:191, No. 1048 (1968).
- WETTER, C. 1971. Potato virus S. C.M.I./A.A.B. Description of Plant Viruses, No. 60.
- WETTER, C. 1972. Potato virus M. C.M.I./A.A.B. Description of Plant Viruses, No. 87.
- WETTER, C., and J. BRANDES. 1956. [Studies on potato virus S.] Cit.:Rev. appl. Mycol. 35:536 (1956).
- WETTER, C., and J. VÖLK. 1960. [Experiments on the transmission of potato virus M and S by *Myzus persicae* Sulz.] Cit.:Rev. appl. Mycol. 40:238 (1961).
- WHITFIELD, P.R., and T.J.V. HIGGINS. 1976. Occurrence of shoot particles in beans infected with the cowpea strain of TMV. I. Purification and characterization of short particles. Virology 71:471-485.

- WHITFIELD, P.R., and S. WILLIAMS. 1963. On the ribonuclease activity associated with tobacco mosaic virus preparations. *Virology* 21:156-161.
- WILDMAN, S.G. 1959. The process of infection and virus synthesis with tobacco mosaic virus and other plant viruses. In "The Viruses", ed. F.M. Burnet and W.M. Stanley vol. 2, pp. 1-31, Academic Press, N.Y.
- WILKINSON, R.E., and F.M. BLODGETT. 1948. *Gomphrena globosa*, a useful plant for quantitative and qualitative work with potato virus X. (Abs.) *Phytopathology* 38:28.
- WILTSHIRE, G.H. 1956. The effect of darkening on the susceptibility of plants to infection with viruses. I. Relation to changes in some organic acids in French bean. *Ann. appl. Biol.* 44:233-248.
- WRIGHT, N.S. 1970. Combined effects of potato viruses X and S on yield of Netted Gem and White Rose potatoes. *Am. Potato J.* 47: 475-478.
- WRIGHT, N.S., and F.C. MELLOR. 1976. A comparison of five clones of virus-free Netted Gem potato from British Columbia and Idaho. *Am. Potato J.* 53:99-103.
- YAMAMOTO, K.R., B.M. ALBERTS, R. BENZINGER, L. LAWHORNE, and G. TREIBER. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. *Virology* 40:734-744.
- YARWOOD, C.E. 1952. Some relations of carbohydrate level of the host plant to plant virus infections. *Am. J. Bot.* 39:119-124.
- YARWOOD, C.E., and A.H. GOLD. 1955. Guar as a local lesion host of potato virus S. *Pl. Dis. Rep.* 39:622.
- YORA, K., and T. TSUCHIZAKI. 1962. [The elimination of latent viruses from potatoes of Danshaku variety by use of tissue culture.] *Cit.:Rev. appl. Mycol.* 42:213 (1963).
- ZACHOS, D.G. 1957. [Studies on the interference between tobacco mosaic virus and potato virus X in the case of complex disease of tomato (streak).] *Cit.:Rev. appl. Mycol.* 37:314 (1958).
- ZADINA, J. 1971. [The susceptibility of the progeny of potato var. 'Saco' to virus S.] *Cit.:Rev. Pl. Path.* 51:330, No. 1801 (1972).

APPENDIX

TABLE 16. Lesion counts: The effect of post-inoculation temperature on the local lesion number of potato virus M in 'Red Kidney' bean.

Expt.	Leaf no.	Temperature ($^{\circ}\text{C}$) ^a			
		17	22	27	32
<i>I</i>	1	52	4	0	0
	2	64	7	0	0
	3	70	7	0	1
	4	52	2	0	0
	5	35	10	0	0
	6	50	4	0	0
	Lesion no./ half-leaf	26.9	2.8	0	0.1
	Standard error	2.5	0.6	0	0.1
<i>II</i>	1	24	8	0	0
	2	25	3	0	0
	3	48	5	0	0
	4	39	7	0	0
	5	10	13	0	0
	6	15	17	2	0
	Lesion no./ half-leaf	13.4	4.4	0.2	0
	Standard error	2.9	1.1	0.2	0

^a Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

TABLE 17. Lesion counts: Infectivity of potato virus M extracted from frozen infected potato leaves in 'Red Kidney' bean at various time intervals after their incubation at 4° or at 24° C.

Half- leaf no.	Time (hr) ^a											
	0		0.5		2		8		24		48	
	Temperature (°C)											
			4	24	4	24	4	24	4	24	4	24
1	19	23	25	82	33	32	15	7	13	5	6	0
2	26	28	12	63	103	49	9	1	10	1	8	0
3	17	24	5	57	9	16	10	5	4	0	5	0
4	44	37	21	71	17	12	10	3	1	0	2	0
5	7	3	12	42	42	30	15	0	4	0	5	0
6	15	12	8	63	35	21	30	0	1	0	1	0
7	4	4	21	67	206	67	10	3	11	1	1	0
8	0	3	7	32	74	33	15	0	5	1	5	0
9	31	37	1	14	83	28	18	5	2	1	1	0
10	30	20	3	3	114	26	31	0	3	1	3	0
11	4	10	3	16	104	36	22	1	2	0	3	0
12	15	16	3	7	193	84	34	2	4	1	2	0
Mean	17.9		10.1	43.1	84.4	36.2	18.3	2.3	5.0	0.9	3.5	0
Standard error	2.5		2.4	8.0	18.6	6.1	2.6	0.7	1.2	0.4	0.7	0
Infectivity index	1.0		0.6	2.4	4.7	2.0	1.0	0.1	0.3	0.1	0.2	0

^a Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

TABLE 18. Lesion counts: Determination of the infectivity of potato virus M in 'Red Kidney' bean at different growth stages of infected 'King Edward' potato plants.

Sample ^a	Days after seeding of tubers	Plant height (cm)		Sampling height (cm)		Lesion no./half-leaf	
		<i>Expt. I</i>	<i>Expt. II</i>	<i>Expt. I</i>	<i>Expt. II</i>	<i>Expt. I</i>	<i>Expt. II</i>
1	22	27.2	18.3	16.5	10.2	62.3	69.3
2	25	35.6	27.2	20.8	19.6	15.8	22.5
3	28	43.2	33.0	34.3	25.4	46.5	32.0
4	31	53.3	43.2	38.6	30.5	6.3	11.0
5	34	59.7	50.8	50.8	36.8	68.3	46.8
6	37	66.8	55.9	54.6	42.4	32.3	23.3
7	40	76.2	63.5	62.2	48.3	79.8	88.0
8	43 ^b	85.1	71.1	66.0	52.6	8.0	14.0
9	46	92.7	80.0	74.9	61.0	24.8	20.5
10	49	97.3	83.8	84.6	73.7	7.8	15.3

^a Inoculum: sap was extracted at a 1:10 (w/v) ratio in 0.057 M phosphate buffer, pH 8.0.

^b Blossom stage.

TABLE 19A. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean. *Expt. 1.*

Inoculation time	Half-leaf no.	Days after inoculation									
		4		5		6		7		9	
		C ^a	T ^b	C	T	C	T	C	T	C	T
0 time	1	18	7	41	57	45	262	47	260	47	257
	2	14	14	36	84	39	304	39	302	40	300
	3	5	3	8	28	9	84	10	91	10	92
	4	8	4	12	22	12	82	12	80	12	79
	5	10	5	24	51	27	189	29	194	29	196
	6	13	18	32	78	35	284	37	308	37	301
	7	7	4	11	33	12	60	12	103	12	113
	8	6	6	10	34	10	80	12	130	12	133
	9	9	10	14	66	14	162	14	166	14	163
	10	10	8	17	45	19	117	21	145	21	144
	11	8	11	15	68	16	200	16	205	16	207
	12	11	6	22	32	24	125	25	151	26	150
	Mean	9.9	8.0	20.2	49.8	21.8	162.4	22.8	177.9	23.0	177.9
	Standard error	1.1	1.3	3.2	6.0	3.5	24.7	3.6	22.5	3.7	21.9

^a C=Control, i.e. 0.33 mg PVM/ml.

^b T=Test, i.e. 0.33 mg PVM+0.40 mg PVS/ml.

TABLE 19A. cont.

Inoculation time (1 hr) ^a	Half- leaf no.	Days after inoculation									
		4		5		6		7		9	
		C	T	C	T	C	T	C	T	C	T
M → S	1	19	18	34	30	36	50	36	52	36	54
	2	24	33	43	55	46	81	50	88	50	86
	3	15	15	29	31	30	52	32	57	32	56
	4	29	23	37	45	40	57	43	60	43	60
	5	31	28	57	41	59	77	62	80	62	82
	6	12	25	27	35	28	55	29	54	29	54
	7	13	11	25	20	25	35	25	37	25	39
	8	16	8	20	17	21	23	23	28	23	27
	9	11	10	14	17	15	22	15	24	15	27
	10	9	8	17	11	18	18	18	23	18	23
	11	10	17	24	27	25	34	27	39	27	37
	12	17	19	31	33	33	60	38	61	38	58
	Mean	17.2	17.9	29.8	30.2	31.3	47.0	33.2	50.3	33.2	50
	Standard error	2.1	2.3	3.4	3.7	3.6	6.0	3.9	6.0	3.9	6
S → M	1	9	7	16	35	16	48	17	60	17	60
	2	6	6	12	29	12	36	12	47	12	46
	3	17	9	31	98	33	140	36	150	36	153
	4	12	8	26	88	27	160	27	173	27	177
	5	5	5	9	25	9	29	9	37	9	36
	6	7	5	10	28	10	36	10	34	10	34
	7	7	4	11	25	11	35	11	47	11	44
	8	8	3	17	34	18	72	20	72	20	69
	9	14	4	25	45	26	110	28	131	28	136
	10	10	4	20	51	20	84	21	90	21	88
	11	6	5	14	39	14	56	15	57	15	58
	12	13	2	20	38	21	60	23	71	23	70
	Mean	9.5	5.2	17.6	44.6	18.1	72.2	19.1	80.1	19.1	81
	Standard error	1.1	0.6	2.1	6.9	2.2	12.5	2.4	13.3	2.4	14

^a Second virus was applied 1 hr after.

TABLE 19A. cont.

Inoculation time (10hr) ^a	Half- leaf no.	Days after inoculation									
		4		5		6		7		9	
		C	T	C	T	C	T	C	T	C	T
M → S	1	11	16	25	22	27	29	29	34	29	34
	2	8	5	20	19	21	21	24	28	25	30
	3	13	6	24	10	26	11	29	19	29	21
	4	9	5	13	22	13	25	15	32	16	34
	5	19	16	39	57	43	62	45	73	46	71
	6	15	20	30	35	33	50	35	69	36	66
	7	15	10	28	21	29	31	30	36	30	39
	8	10	13	18	23	18	25	19	31	19	30
	9	10	4	19	9	19	17	20	28	20	29
	10	9	6	16	10	16	23	17	22	17	23
	11	6	6	9	17	10	20	10	23	10	24
	12	7	4	11	10	11	15	12	17	12	17
	Mean	11.0	9.3	21.0	21.3	22.2	27.4	23.8	34.3	24.1	34.8
	Standard error	1.1	1.6	2.5	3.9	2.8	4.3	3.0	5.2	3.0	4.9
S → M	1	14	5	20	21	21	39	21	40	21	40
	2	6	13	9	29	11	49	11	57	11	58
	3	16	26	26	65	26	94	28	104	30	106
	4	22	9	41	34	41	59	44	65	46	64
	5	25	17	32	39	33	48	35	54	36	56
	6	12	26	23	42	23	50	26	49	27	48
	7	19	30	34	88	35	106	37	110	39	108
	8	15	20	21	59	21	89	22	88	23	87
	9	13	7	24	23	26	31	26	39	26	38
	10	9	7	19	30	19	50	20	55	22	57
	11	7	8	10	44	11	63	12	67	12	70
	12	11	9	18	33	20	64	20	65	20	66
	Mean	14.1	14.8	23.1	42.3	23.9	61.8	25.2	66.1	26.1	66.5
	Standard error	1.7	2.6	2.7	5.7	2.6	6.7	2.8	6.7	3.0	6.7

^a The second virus was applied 10 hr after.

TABLE. 19B. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with purified potato virus M (PVM) in 'Red Kidney' bean.
Expt. II.

Inocu- lation time	Half- leaf no.	Days after inoculation											
		4		5		6		7		8		9	
		C ^a	T ^b	C	T	C	T	C	T	C	T	C	T
0 time	1	4	6	18	59	20	84	25	99	25	99	26	99
	2	3	8	8	37	14	59	18	65	18	71	18	68
	3	4	12	12	64	13	85	23	99	23	108	23	112
	4	4	15	19	51	20	78	30	94	30	105	32	108
	5	7	20	24	116	27	140	38	171	39	182	39	180
	6	13	29	40	111	44	178	55	178	56	188	56	188
	7	0	9	8	23	9	33	12	43	12	50	13	50
	8	3	9	11	29	11	40	20	49	20	57	21	60
	9	9	18	21	93	21	127	27	145	31	148	31	148
	10	10	34	32	109	35	152	40	183	40	200	43	202
	11	8	8	19	50	21	64	29	79	29	90	29	90
	12	7	9	17	44	17	72	23	92	23	94	23	94
Mean		6.0	14.8	19.1	65.5	21.0	92.7	28.3	108.1	28.8	116.0	29.5	117
Standard error		1.1	2.6	2.8	9.6	2.9	13.3	3.3	14.3	3.4	14.8	3.4	15

^a C = Control, i.e. 0.56 mg PVM/ml.

^b T = Test, i.e. 0.56 mg PVM + 0.80 mg PVS/ml.

TABLE 198. cont.

Inocu- lation time (1 hr) ^a	Half- leaf no.	Days after inoculation											
		4		5		6		7		8		9	
		C	T	C	T	C	T	C	T	C	T	C	T
M→S	1	2	3	9	25	17	37	20	38	20	39	20	40
	2	2	4	10	29	19	39	23	46	23	46	23	47
	3	5	7	24	70	42	105	45	106	48	106	48	105
	4	3	8	14	94	23	134	26	134	34	135	34	135
	5	1	3	6	29	14	39	18	46	20	49	20	50
	6	8	3	20	47	31	61	33	67	33	71	32	70
	7	3	3	10	47	21	64	25	69	25	74	25	73
	8	7	1	19	36	30	66	34	68	34	67	33	66
	9	3	8	11	81	22	136	24	153	24	160	24	160
	10	2	7	9	72	21	107	25	115	25	122	25	122
	11	1	1	5	20	13	37	15	41	15	46	15	44
	12	1	2	4	19	10	37	12	46	12	47	12	45
Mean		3.2	4.2	11.8	47.4	21.9	71.8	25.0	77.4	26.1	80.2	25.9	79.8
Standard error		0.7	0.8	1.8	7.4	2.6	11.1	2.6	11.4	2.8	11.7	2.8	11.8
S→M	1	8	11	20	52	31	89	40	99	42	107	43	112
	2	3	9	14	86	20	142	30	148	31	151	32	150
	3	1	10	5	79	7	115	9	121	13	124	13	120
	4	3	11	6	62	10	73	14	87	17	97	17	99
	5	0	7	5	35	9	42	11	52	11	53	11	52
	6	3	8	7	25	11	28	13	40	13	41	13	42
	7	6	13	22	61	26	87	33	89	36	95	36	94
	8	2	16	13	88	18	105	23	116	25	125	25	120
	9	1	13	5	60	8	73	10	87	15	93	15	93
	10	3	9	11	44	13	46	20	58	23	60	23	60
	11	4	8	12	44	19	45	24	46	24	49	24	49
	12	2	6	8	43	14	44	21	55	21	55	21	54
Mean		3.0	10.1	10.7	56.6	15.5	74.1	20.7	83.2	22.6	87.5	22.8	87.1
Standard error		0.6	0.8	1.7	5.8	2.2	10.1	2.9	9.8	2.8	10.3	2.9	10.1

^a The second virus was applied 1 hr after.

TABLE 198. cont.

Inocu- lation time (5 hr) ^a	Half- leaf no.	Days after inoculation											
		4		5		6		7		8		9	
		C	T	C	T	C	T	C	T	C	T	C	T
M→S	1	12	16	29	30	32	53	34	54	35	53	36	52
	2	8	12	13	32	15	43	18	44	20	44	20	42
	3	3	7	5	10	7	18	11	21	11	23	11	24
	4	3	11	7	19	10	38	13	39	14	39	14	39
	5	4	9	6	18	9	22	10	24	10	24	10	24
	6	2	10	4	12	7	15	8	18	8	17	8	22
	7	8	17	20	39	24	49	27	50	27	54	27	52
	8	9	15	12	18	14	31	16	39	16	40	16	40
	9	3	10	7	14	9	21	9	25	9	23	9	22
	10	4	9	8	14	11	20	14	19	14	21	14	23
	11	11	20	26	36	29	51	31	53	31	57	31	57
	12	7	7	14	26	18	42	21	45	21	45	21	44
Mean		6.2	11.9	12.6	22.3	15.4	33.6	17.7	35.9	18.0	36.7	18.1	37
Standard error		1.0	1.2	2.4	2.9	2.5	4.1	2.5	4.0	2.6	4.2	2.6	4
S→M	1	4	7	15	49	31	72	35	85	35	93	35	97
	2	2	3	10	45	16	67	18	74	21	78	23	78
	3	3	5	8	32	11	56	14	60	16	63	18	62
	4	6	6	14	50	29	78	29	78	29	78	29	78
	5	1	2	9	16	11	25	12	25	12	27	13	27
	6	2	4	12	14	20	22	22	28	22	34	22	33
	7	5	5	20	40	41	72	42	77	43	88	43	89
	8	7	10	23	75	39	94	43	105	45	114	45	113
	9	3	2	10	19	18	28	18	35	18	36	18	35
	10	1	3	7	12	9	24	9	27	10	31	10	32
	11	0	2	6	15	10	19	10	20	10	23	10	22
	12	1	5	5	16	8	20	9	25	9	28	9	29
Mean		2.9	4.5	11.6	31.9	20.3	48.1	21.8	53.3	22.5	57.8	22.9	58
Standard error		0.6	0.7	1.6	5.8	3.4	8.0	3.6	8.6	3.7	9.1	3.6	9

^a The second virus was applied 5 hr after.

TABLE 19B. cont.

Inocu- lation time (10 hr) ^a	Half- leaf no.	Days after inoculation											
		4		5		6		7		8		9	
		C	T	C	T	C	T	C	T	C	T	C	T
M→S	1	3	3	8	16	11	20	15	24	15	25	15	25
	2	1	6	7	13	10	18	10	26	12	26	12	26
	3	3	10	12	20	16	36	20	49	25	53	26	53
	4	9	8	15	32	20	47	23	50	32	52	32	52
	5	3	1	5	4	8	11	8	12	9	12	9	12
	6	1	3	4	7	6	11	6	14	6	16	6	16
	7	4	4	9	15	15	20	16	23	18	28	20	28
	8	4	1	10	8	12	17	12	17	12	21	12	21
	9	4	1	7	8	9	13	9	18	11	22	11	21
	10	1	3	5	8	7	13	9	17	10	17	10	17
	11	3	6	10	15	12	24	13	26	15	27	15	26
	12	5	5	14	12	17	22	19	27	23	27	23	27
	Mean	3.4	4.3	8.8	13.2	11.9	21.0	13.3	25.3	15.7	27.2	15.9	27
	Standard error	0.6	2.9	1.0	2.2	1.3	3.1	1.5	3.6	2.2	3.7	2.2	4
S→M	1	4	2	20	25	22	40	22	56	24	62	24	61
	2	5	3	20	20	20	36	20	36	21	41	21	41
	3	2	1	11	12	12	19	12	26	12	26	12	26
	4	2	0	8	7	9	16	9	24	10	27	10	27
	5	2	3	14	32	15	45	15	56	17	57	17	57
	6	3	2	19	27	20	30	20	42	21	43	21	43
	7	0	1	6	9	7	18	7	21	7	24	7	24
	8	2	0	9	8	9	15	9	17	9	17	9	17
	9	2	1	18	17	21	24	21	34	23	43	23	43
	10	6	2	24	30	30	57	30	64	34	64	34	63
	11	2	0	10	5	12	15	12	21	14	21	14	21
	12	2	1	7	15	7	26	8	29	9	29	10	29
	Mean	2.7	1.3	13.8	17.3	15.3	28.4	15.4	35.5	16.8	37.8	16.8	38
	Standard error	0.5	0.3	1.8	2.7	2.1	3.9	2.1	4.5	2.3	4.7	2.3	5

^a The second virus was applied 10 hr after.

TABLE 20A. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with potato virus S (PVS) in *Chenopodium quinoa*. Expt. I.

Inoculation time	Half-leaf no.	Days after inoculation									
		6		7		8		9		10	
		C ^a	T ^b	C	T	C	T	C	T	C	T
0 time	1	0	1	3	11	11	29	30	80	30	79
	2	0	1	4	15	19	43	38	155	39	155
	3	2	4	11	38	21	118	58	277	60	270
	4	2	3	9	31	32	114	79	222	81	227
	5	0	0	4	11	8	39	21	88	21	89
	6	0	1	5	14	14	42	33	107	33	111
	7	0	3	7	18	14	44	30	153	30	160
	8	1	5	7	39	20	95	71	201	71	199
	9	0	3	3	22	9	45	29	99	29	98
	10	0	4	5	33	31	82	77	197	79	195
	11	3	7	12	60	34	135	106	284	105	280
	12	1	3	5	31	29	63	50	131	52	133
Mean		0.8	2.9	6.3	26.9	20.2	70.8	51.8	166.2	52.5	166.3
Standard error		0.3	0.6	0.9	4.2	2.7	10.6	7.7	20.3	7.7	19.8

^a C=Control, i.e. 0.08 mg PVS/ml.

^b T=Test, i.e. 0.08 mg PVS + 1.67 mg PVM/ml.

TABLE 20A cont.

Inoculation time (1 hr) ^a	Half- leaf no.	Days after inoculation									
		6		7		8		9		10	
		C	T	C	T	C	T	C	T	C	T
S → M	1	1	1	6	12	18	37	31	89	31	88
	2	0	3	9	13	25	43	47	89	49	90
	3	1	1	8	11	28	46	54	100	56	101
	4	4	5	23	22	43	91	103	178	104	175
	5	0	0	5	7	10	20	20	44	22	48
	6	0	2	5	13	10	32	22	54	24	57
	7	0	1	4	10	16	23	29	68	30	68
	8	1	4	11	16	22	45	34	90	34	89
	9	0	3	7	11	12	33	25	85	25	87
	10	2	8	12	45	29	84	58	150	58	147
	11	1	4	12	22	31	65	69	115	70	114
	12	3	10	19	51	40	94	99	203	100	200
	Mean	1.1	3.5	10.1	19.4	23.7	51.1	49.3	105.4	50.3	105
	Standard error	0.4	0.9	1.7	4.1	3.2	7.5	8.3	14.0	8.3	13
M → S	1	0	2	2	13	7	39	17	56	18	58
	2	1	0	2	9	8	28	18	70	18	71
	3	0	4	3	15	11	31	30	89	30	88
	4	0	4	2	17	10	38	28	71	28	70
	5	0	2	2	11	8	31	15	62	16	62
	6	1	6	3	20	12	50	34	103	34	101
	7	1	6	10	28	27	55	60	116	60	114
	8	2	18	7	58	17	90	40	192	40	190
	9	1	4	4	22	24	50	50	100	50	99
	10	2	8	6	36	21	70	46	154	46	154
	11	2	10	8	38	31	82	75	224	75	221
	12	2	15	10	67	30	137	68	248	70	245
	Mean	1.0	6.6	4.9	27.8	17.2	58.4	40.1	123.8	40.4	123
	Standard error	0.3	1.6	0.9	5.4	2.6	9.2	5.8	19.0	5.8	19

^a The second virus was applied 1 hr after.

TABLE 20A. cont.

Inoculation time (10 hr) ^a	Half- leaf no.	Days after inoculation									
		6		7		8		9		10	
		C	T	C	T	C	T	C	T	C	T
S → M	1	0	0	0	3	5	10	15	20	15	20
	2	0	0	1	4	7	13	18	35	18	36
	3	0	0	0	6	7	13	21	31	22	30
	4	2	2	5	10	15	30	58	80	58	79
	5	0	0	0	2	6	7	16	18	16	19
	6	1	0	7	7	19	18	52	57	52	60
	7	2	1	10	13	29	45	75	102	75	100
	8	0	0	4	11	17	35	62	77	61	74
	9	0	0	0	2	6	9	20	20	20	24
	10	0	0	1	4	8	10	23	30	23	30
	11	1	1	4	3	13	13	36	33	36	33
	12	0	0	2	6	19	15	46	53	46	52
	Mean	0.5	0.3	2.8	5.9	12.6	18.2	36.8	46.4	36.8	46
	Standard error	0.2	0.2	0.9	1.1	2.1	3.5	6.1	7.9	6.0	8
M → S	1	0	0	1	9	4	26	13	40	13	45
	2	0	0	0	10	5	32	15	64	16	65
	3	0	0	2	10	10	41	20	93	23	92
	4	1	1	6	18	18	67	60	134	60	131
	5	0	0	1	7	5	33	14	64	14	68
	6	0	0	2	5	7	33	17	73	17	73
	7	0	1	4	16	15	64	32	140	34	137
	8	1	1	4	20	17	73	45	150	45	148
	9	0	0	1	10	7	39	16	77	16	76
	10	0	0	1	5	6	20	23	60	23	60
	11	1	2	5	32	20	100	54	168	54	163
	12	0	0	1	8	7	31	25	70	25	72
	Mean	0.3	0.4	2.3	12.5	10.1	46.6	27.8	94.4	28.3	94
	Standard error	0.1	0.2	0.6	2.3	1.7	6.9	4.7	12.2	4.7	11

^a The second virus was applied 10 hr after.

TABLE 20B. Lesion counts: Increment curves of local lesions obtained after pre- and post-inoculation treatments with potato virus S (PVS) in *Chenopodium quinoa*.
Expt. II.

Inocu- lation time	Half- leaf no.	Days after inoculation											
		6		7		8		9		10		11	
		C ^a	T ^b	C	T	C	T	C	T	C	T	C	T
0 time	1	2	7	4	17	14	51	37	106	57	168	57	165
	2	0	8	2	19	8	66	20	150	37	231	37	227
	3	0	1	0	6	7	17	16	52	30	99	30	100
	4	2	0	3	4	6	15	13	40	27	73	27	78
	5	1	3	3	9	10	33	27	92	41	148	41	143
	6	1	2	2	7	9	30	19	73	34	109	34	106
	7	1	2	3	5	7	22	15	63	28	84	28	84
	8	0	1	1	5	5	15	9	46	19	73	19	76
	9	2	5	3	14	15	45	33	107	53	178	53	175
	10	3	4	4	14	20	60	47	116	79	225	78	222
	11	0	2	1	6	6	16	15	48	22	89	22	88
	12	0	2	1	4	4	14	12	25	20	55	20	57
Mean		1.0	3.1	2.3	9.2	9.3	32.0	22.0	76.5	37.3	127.7	37.2	127
Standard error		0.3	0.7	0.4	1.6	1.4	5.5	3.4	10.9	5.2	17.5	5.1	17

^a C = Control, i.e. 0.16 mg PVS/ml.

^b T = Test, i.e. 0.16 mg PVS + 2.78 mg PVM/ml.

TABLE 20B. cont.

Inoculation time (1 hr) ^a	Half- leaf no.	Days after inoculation											
		6		7		8		9		10		11	
		C	T	C	T	C	T	C	T	C	T	C	T
S → M	1	0	2	1	7	6	13	13	25	26	38	26	40
	2	0	3	2	9	8	16	19	31	28	48	28	51
	3	0	0	1	7	5	21	11	49	24	64	24	65
	4	1	0	3	4	9	13	21	30	32	41	32	40
	5	3	9	8	23	21	60	47	130	77	170	79	167
	6	2	12	6	28	14	45	31	110	64	133	67	130
	7	2	5	5	12	10	29	27	70	38	82	38	80
	8	1	4	4	10	19	37	30	72	45	108	47	106
	9	3	3	5	11	17	40	32	95	50	106	53	110
	10	2	2	3	6	14	31	27	77	36	89	37	87
	11	0	1	2	5	8	14	15	30	19	40	19	44
	12	1	3	3	8	10	19	20	40	30	59	30	60
Mean		1.3	3.7	3.6	10.8	11.8	28.2	24.4	63.3	39.1	81.5	40.0	82
Standard error		0.3	1.0	0.6	2.1	1.5	4.3	2.9	10.1	5.0	12.0	5.3	12
M → S	1	0	0	0	3	4	14	13	36	22	87	22	86
	2	0	0	0	5	4	28	12	53	29	121	31	119
	3	0	2	3	18	8	39	28	89	54	209	55	204
	4	1	1	4	10	11	33	32	95	60	196	62	193
	5	0	0	1	9	6	27	15	49	25	82	26	83
	6	0	1	2	12	7	25	17	48	31	96	31	94
	7	0	1	2	11	7	21	15	44	21	63	22	65
	8	0	0	0	4	7	13	17	31	20	79	21	81
	9	0	0	0	8	8	20	20	47	38	77	38	80
	10	0	1	1	7	13	24	33	67	43	157	43	155
	11	0	0	1	4	4	11	9	29	14	51	14	54
	12	0	0	0	4	3	10	8	28	15	42	15	45
Mean		0.1	0.5	1.2	7.9	6.8	22.1	18.3	51.3	31.0	105.0	31.7	105
Standard error		0.1	0.2	0.4	1.3	0.9	2.6	2.4	6.4	4.3	15.8	4.4	15

^aThe second virus was applied 1 hr after.

TABLE 20B. cont.

Inocu- lation time (5 hr) ^a	Half- leaf no.	Days after inoculation											
		6		7		8		9		10		11	
		C	T	C	T	C	T	C	T	C	T	C	T
S → M	1	3	1	5	2	10	30	21	86	54	98	53	95
	2	0	0	0	0	5	20	11	50	29	67	29	68
	3	0	0	1	0	3	10	7	40	27	69	27	67
	4	1	0	3	1	8	21	14	66	44	88	44	86
	5	0	0	1	0	4	15	10	45	30	70	30	70
	6	0	1	1	2	3	20	7	60	24	67	24	66
	7	0	0	1	1	3	14	8	30	16	40	16	42
	8	0	0	0	0	2	7	5	21	14	31	14	31
	9	0	0	1	1	6	10	13	40	41	75	41	76
	10	0	0	0	0	2	8	5	35	20	50	20	51
	11	1	0	3	2	8	24	17	72	60	88	60	89
	12	2	1	5	3	12	31	25	103	77	111	75	110
Mean		0.6	0.3	1.7	1.0	5.5	17.5	11.9	54.0	36.3	71.2	36.1	71
Standard error		0.3	0.1	0.5	0.3	1.0	2.4	1.8	7.0	5.6	6.7	5.5	7
M → S	1	1	0	2	0	4	13	11	53	21	63	21	64
	2	0	1	1	2	4	22	12	69	30	90	30	88
	3	0	2	0	4	3	12	9	49	15	60	15	60
	4	2	0	3	1	6	15	10	58	27	69	27	70
	5	2	3	4	5	12	35	27	91	50	123	50	120
	6	1	1	2	3	10	40	24	111	61	111	60	110
	7	1	1	2	2	6	23	14	69	39	82	39	81
	8	0	0	1	1	5	23	14	67	32	74	32	75
	9	0	0	0	1	3	14	8	47	16	58	16	60
	10	2	1	3	2	9	30	17	87	42	100	42	101
	11	1	0	2	1	5	19	10	59	14	63	14	63
	12	0	0	0	0	2	11	6	32	10	47	10	48
Mean		0.8	0.8	1.7	1.8	5.8	21.4	13.5	66.0	29.8	78.3	29.7	78
Standard error		0.2	0.3	0.4	0.4	0.9	2.7	1.8	6.3	4.6	6.7	4.5	6

^a The second virus was applied 5 hr after.

TABLE 20B. cont.

Inoculation time (10 hr) ^a	Half- leaf no.	Days after inoculation											
		6		7		8		9		10		11	
		C	T	C	T	C	T	C	T	C	T	C	T
S → M	1	0	0	2	10	7	22	13	41	32	70	32	69
	2	0	0	1	4	3	10	8	23	27	37	27	40
	3	1	0	2	3	4	18	7	33	17	48	19	47
	4	0	0	0	7	4	17	10	30	27	55	27	58
	5	0	1	4	19	9	34	27	71	42	88	42	85
	6	1	0	3	19	7	40	17	91	50	131	50	128
	7	0	0	0	2	2	9	4	16	14	29	14	30
	8	0	0	0	2	2	9	4	18	13	32	13	32
	9	0	0	0	7	3	12	9	23	16	37	19	37
	10	0	0	0	5	3	13	7	26	19	48	19	48
	11	1	0	2	6	5	20	11	41	32	66	32	64
	12	0	0	0	12	6	25	13	46	30	73	30	74
Mean		0.3	0.1	1.2	8.0	4.6	19.1	10.8	38.3	26.6	59.5	27.0	59
Standard error		0.1	0.1	0.4	1.7	0.7	2.9	1.8	6.5	3.3	8.4	3.2	8
M → S	1	0	0	1	2	7	15	13	50	25	66	25	66
	2	0	1	0	3	4	13	9	47	19	54	19	54
	3	0	0	1	9	5	24	10	77	31	95	30	93
	4	0	2	2	6	5	29	12	110	32	130	32	127
	5	0	1	1	5	6	9	12	39	18	58	18	57
	6	0	0	0	3	3	12	8	44	16	55	16	58
	7	0	0	0	4	3	16	9	52	20	61	20	62
	8	0	0	2	3	8	20	13	67	27	79	27	78
	9	0	0	0	1	2	7	6	25	12	40	12	42
	10	0	1	0	2	2	7	7	23	15	29	15	30
	11	0	1	3	5	12	21	33	54	43	79	41	79
	12	0	0	2	10	9	31	24	114	50	144	50	140
Mean		0	0.5	1.0	4.4	5.5	17.0	13.0	58.5	25.7	74.2	25.4	74
Standard error		0	0.2	0.3	0.8	0.9	2.3	2.3	8.4	3.4	9.9	3.3	9

^a The second virus was applied 10 hr after.

TABLE 21. Lesion counts: The dilution curves of the infectivity of ribonucleic acids respectively isolated from potato virus M (PVM) and potato virus S (PVS) in 'Red Kidney' bean (RKB) and *Chenopodium quinoa*.

Local lesion host	Half-leaf no.	Dilution ^a				
		0 ^b	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
RKB	1	13	0	0	0	0
	2	4	4	1	0	0
	3	9	1	0	0	0
	4	11	7	1	0	0
	5	12	4	2	0	0
	6	6	4	0	0	0
	7	3	1	0	0	0
	8	8	5	1	0	0
	9	7	3	1	0	0
	10	16	2	2	0	0
	11	12	6	0	0	0
	12	10	3	2	0	0
	Mean	9.3	3.3	0.8	0	0
	Standard error	1.1	0.6	0.2	0	0
<i>C. quinoa</i>	1	98	66	30	5	0
	2	132	50	17	1	0
	3	177	70	22	3	0
	4	142	41	16	6	0
	5	180	118	13	2	0
	6	216	96	26	5	0
	7	304	44	14	1	0
	8	264	68	9	4	0
	9	152	62	21	0	0
	10	134	80	13	3	0
	11	124	53	15	4	0
	12	165	38	11	2	0
	Mean	174.0	65.5	17.3	3.0	0
	Standard error	17.4	6.8	1.8	0.5	0

^a By using 0.057 M phosphate buffer, pH 8.0.

^b Concentration: 50 A units at 258 nm.

TABLE 22. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus S (PVS) on the infectivity of potato virus M (PVM) - ribonucleic acid (RNA) in 'Red Kidney' bean.

Expt.	Half-leaf no.	Control ^a	Test (<i>PVM-RNA</i> + <i>PVS</i>)				
		45	45 ^b 2.4 ^c	45 0.24	45 0.024	45 0.0024	45 0.00024
<i>I</i>	1	11	3	9	6	11	18
	2	5	0	4	4	9	10
	3	13	2	3	11	8	8
	4	7	2	5	8	6	9
	5	9	4	3	5	7	14
	6	10	3	6	12	10	6
	7	18	4	2	7	17	15
	8	8	5	7	6	5	12
	9	6	3	4	5	9	7
	10	15	1	5	9	14	10
	11	7	2	5	4	8	8
	12	13	2	4	7	10	9
	Mean	10.2	2.6	4.8	7.0	9.5	10.5
Standard error	1.1	0.4	0.6	0.8	1.0	1.0	
<i>II</i>		35	35 ^b 1.6 ^c	35 0.16	35 0.016	35 0.0016	35 0.00016
	1	5	2	2	6	5	6
	2	8	1	5	10	8	10
	3	9	0	3	3	11	13
	4	7	1	6	9	9	5
	5	7	1	3	3	7	4
	6	6	2	5	4	7	7
	7	12	4	4	5	9	6
	8	5	3	2	5	5	7
	9	3	1	1	8	6	6
	10	10	5	4	6	4	10
	11	9	3	3	5	8	5
	12	8	4	4	7	4	8
	Mean	7.4	2.3	3.5	5.9	6.9	7.3
	Standard error	0.7	0.5	0.4	0.7	0.6	0.8

^a PVM-RNA only (4 units at 258 nm).

^b 4 units of PVM-RNA at 258 nm.

^c PVS (mg/ml).

TABLE 23. Lesion counts: Effect of endogenous ribonuclease associated with intact potato virus M (PVM) on the infectivity of potato virus S (PVS)-ribonucleic acid (RNA) in *Chenopodium quinoa*.

Expt.	Half-leaf no.	Control ^a	Test (PVS-RNA + PVM)			
			10 ^b 1.1 ^c	10 0.11	10 0.011	10 0.0011
I	1	129	58	102	116	142
	2	54	26	87	90	66
	3	94	20	44	42	62
	4	67	27	65	74	72
	5	104	60	95	95	122
	6	118	27	74	52	132
	7	62	26	45	64	59
	8	97	35	34	65	69
	9	78	41	68	79	96
	10	78	45	50	81	50
	11	97	53	63	68	91
	12	90	39	38	61	64
	Mean	89.0	38.1	63.8	73.9	85.4
	Standard error	6.5	3.9	6.5	5.8	9.0
II		6	6 ^b 0.7 ^c	6 0.07	6 0.007	6 0.0007
	1	48	17	50	34	55
	2	95	36	66	66	99
	3	118	14	31	110	131
	4	130	19	28	88	69
	5	42	13	46	58	48
	6	43	45	36	44	83
	7	68	30	80	47	38
	8	77	56	77	58	45
	9	50	23	94	90	40
	10	81	50	41	71	70
	11	44	40	33	69	108
	12	58	32	59	98	90
	Mean	71.2	31.3	53.4	69.4	73.0
	Standard error	8.7	4.2	6.3	6.7	8.6

^a PVS RNA only (4 units at 258 nm).

^b 4 units of PVS RNA at 258 nm.

^c PVM (mg/ml).

B30171